

## Miller-Saunders, Kristi

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**From:** Malcolm, Gabrielle  
**Sent:** January-04-19 9:18 AM  
**To:** [REDACTED] Struthers, Alistair; Waddington, Zac; Miller-Saunders, Kristi; Gagne, Nellie; 'Bruneau, Nathalie (CFIA/ACIA) (nathalie.bruneau@canada.ca)'; 'myron.roth@gov.bc.ca'; [REDACTED]  
**Cc:** Olivier, Gilles; 'Craig Stephen'; Kristmanson, James  
**Subject:** Parsons, Jay; Burgetz, Ingrid  
DFO PRV CSAS - January 28-30 Vancouver, BC

Dear Steering Committee Members,

[REDACTED]

**Please note that the schedule for the PRV CSAS meeting has been revised, and will now incorporate an additional half day on Monday, January 28<sup>th</sup> beginning at 1PM and will go until 5PM on Wednesday, January 30<sup>th</sup>.** It would be greatly appreciated if these meeting times be kept in mind when making travel arrangements.

The meeting will be held at the Delta Hotels Vancouver Downtown Suites (550 West Hastings Street, Vancouver, BC). If you are interested in booking a guestroom at the hotel during the period of January 27<sup>th</sup> – 31<sup>st</sup>, please use the following link to access our group discounted nightly rate (\$199): <https://www.marriott.com/event-reservations/reservation-link.mi?id=1546562104073&key=GRP&app=resvlink>

Please note that we have a limited number of guestrooms reserved at this rate and that the discount is only available if you book by Monday, January 14<sup>th</sup>.

If you have any questions or concerns, please do not hesitate to contact me.

Kind regards,

**Gabrielle Malcolm**

Science Advisor, Aquaculture Regulatory Sciences, Aquaculture, Biotechnology and Aquatic Animal Health Science Branch

Fisheries and Oceans Canada / Government of Canada  
[gabrielle.malcolm@dfo-mpo.gc.ca](mailto:gabrielle.malcolm@dfo-mpo.gc.ca) / T: 613-949-7466

Conseillère des sciences, sciences de réglementation de l'aquaculture, Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques  
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## Miller-Saunders, Kristi

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**From:** Malcolm, Gabrielle  
**Sent:** January-08-19 11:44 AM  
**To:** Miller-Saunders, Kristi; [REDACTED] Struthers, Alistair; Waddington, Zac;  
Gagne, Nellie; 'Bruneau, Nathalie (CFIA/ACIA) (nathalie.bruneau@canada.ca)';  
'myron.roth@gov.bc.ca'; [REDACTED]  
Olivier, Gilles; 'Craig Stephen'; Kristmanson, James  
**Cc:** Parsons, Jay; Burgetz, Ingrid  
**Subject:** RE: DFO PRV CSAS Steering Committee Meeting

Hi Kristi,

I will provide a report to SC members which will include the names of all persons we invited to participate as either a formal reviewer or meeting participant and will highlight those that have accepted our invitation. This will be distributed with the meeting materials before our next call.

Please note that Espen Rimstad has accepted our invitation and will be attending the peer-review meeting. Oystein Wessel will not be attending the meeting, but has accepted to coordinate in a joint review with Dr. Rimstad.

I hope this answers your questions. There will be an opportunity to discuss further on our next call.

Kind regards,

### Gabrielle Malcolm

Science Advisor, Aquaculture Regulatory Sciences, Aquaculture, Biotechnology and Aquatic Animal Health Science  
Branch  
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**From:** Miller-Saunders, Kristi  
**Sent:** January 8, 2019 11:44 AM  
**To:** Malcolm, Gabrielle; [REDACTED] Struthers, Alistair; Waddington, Zac; Gagne, Nellie; 'Bruneau, Nathalie  
(CFIA/ACIA) (nathalie.bruneau@canada.ca)'; 'myron.roth@gov.bc.ca'; [REDACTED]  
[REDACTED]; Olivier, Gilles; 'Craig Stephen'; Kristmanson, James  
**Cc:** Parsons, Jay; Burgetz, Ingrid  
**Subject:** RE: DFO PRV CSAS Steering Committee Meeting

Hello Gabrielle,

Can we possibly be provided with the list of reviewers and participants that were invited for the CSAS. I received the ranking but it was unclear how many people from each category were to be invited. [REDACTED]

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s.21(1)(b)

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Thanks,  
Kristi

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

**From:** Malcolm, Gabrielle

**Sent:** January-08-19 6:47 AM

**To:** [REDACTED] Struthers, Alistair; Waddington, Zac; Miller-Saunders, Kristi; Gagne, Nellie; 'Bruneau, Nathalie (CFIA/ACIA) (nathalie.bruneau@canada.ca)'; 'myron.roth@gov.bc.ca'; '[REDACTED]'; Olivier, Gilles; 'Craig Stephen'; Kristmanson, James

**Cc:** Parsons, Jay; Burgetz, Ingrid

**Subject:** DFO PRV CSAS Steering Committee Meeting

Dear Steering Committee members,

We are proposing to have a third and final Steering Committee meeting before the PRV CSAS at the end of the month to review the CSAS Agenda and provide an update on attending meeting participants and formal reviewers.

Please provide us with your availability for a 1-hour call at the following link:

<https://doodle.com/poll/kwy2sqxnt653emxc>. It would be greatly appreciated if you could participate in this poll by end of day today.

If you have any questions or concerns, please do not hesitate to contact me.

Kind regards,

**Gabrielle Malcolm**

Science Advisor, Aquaculture Regulatory Sciences, Aquaculture, Biotechnology and Aquatic Animal Health Science Branch

Fisheries and Oceans Canada / Government of Canada

[gabrielle.malcolm@dfo-mpo.gc.ca](mailto:gabrielle.malcolm@dfo-mpo.gc.ca) / T: 613-949-7466

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## Miller-Saunders, Kristi

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**From:** Fenton, AJ  
**Sent:** January-09-19 1:27 PM  
**To:** Miller-Saunders, Kristi  
**Cc:** MacDougall, Lesley  
**Subject:** RE: BC Aquaculture All-Staff Retreat 2019  
**Attachments:** BCARP All-Staff Meeting Agenda 2019.doc

Hi Kristi –

Here you go!

Thanks,

### A.J. Fenton

Data Systems Coordinator, Aquaculture Programs  
Fisheries and Oceans Canada, Pacific Region  
[AJ.Fenton@dfo-mpo.gc.ca](mailto:AJ.Fenton@dfo-mpo.gc.ca) / Tel: 604-666-9364

Coordinateur de systèmes de données, programmes d'aquaculture  
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**From:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Sent:** January 9, 2019 12:38  
**To:** Fenton, AJ <AJ.Fenton@dfo-mpo.gc.ca>  
**Cc:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Subject:** RE: BC Aquaculture All-Staff Retreat 2019

I accidentally pushed "tentative" when I really wanted to say I can't open the hyperlink. Can you possibly send the file with the agenda?

Thanks,  
Kristi

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)



-----Original Appointment-----

**From:** Fenton, AJ

**Sent:** January-09-19 11:03 AM

**To:** Fenton, AJ; Miller-Saunders, Kristi; Higgins, Mark; Backe, Nicole; Ballard, Michael; Barry, Melanie; Blasco, Nathan; Buhr, Val; Choi, Shirley; Delaney, Paula; Diamond, Maria; Doucette, Claire; Fenton, AJ; Hedderson, Lisa; Jensen, Neil; Jepps, Shelley; Johansson, Todd; Kosmider, Gabrielle; Lim, Susan; MacDonald, Cristopher; MacDougall, Lesley; Maley, Shelley; Manchester, Howie; Manning, Michelle; Marrie, Christopher; McConnachie, Sarah; McCorquodale, Brenda; Meadows, Shelley; Metcalf, Vanessa; Mollins, Jennifer; Patirana, Anoma; Pauls, Guinevere; Paylor, Adrienne; Plummer, Greg; Rainer, Michelle; Sandberg, Krista; Scott, Melinda; Shaw, Kerra; Squires, Angela; Struthers, Alistair; Taekema, Bernie John; Tomlinson, Daniel; Turunen, Ari; Van Pelt, Ginny; Verkaik, Katie; Waddington, Zac; Walde, Kirsty; Walker, Monica; Webb, Allison; Wilkinson, Davida; Youssef, Andre; Parenteau, Jennifer; Nielsen, Ingrid; MacKenzie, Julia; Thomson, Andrew; Backe, Nicole; Ballard, Michael; Barry, Melanie; Blasco, Nathan; Buhr, Val; Choi, Shirley; Delaney, Paula; Diamond, Maria; Doucette, Claire; Hedderson, Lisa; Jensen, Neil; Jepps, Shelley; Johansson, Todd; Kosmider, Gabrielle; Lim, Susan; MacDonald, Cristopher; MacDougall, Lesley; Maley, Shelley; Manchester, Howie; Manning, Michelle; Marrie, Christopher; McConnachie, Sarah; McCorquodale, Brenda; Meadows, Shelley; Metcalf, Vanessa; Mollins, Jennifer; Patirana, Anoma; Pauls, Guinevere; Paylor, Adrienne; Plummer, Greg; Rainer, Michelle; Sandberg, Krista; Scott, Melinda; Shaw, Kerra; Squires, Angela; Struthers, Alistair; Taekema, Bernie John; Tomlinson, Daniel; Turunen, Ari; Van Pelt, Ginny; Verkaik, Katie; Waddington, Zac; Walde, Kirsty; Walker, Monica; Webb, Allison; Wilkinson, Davida; Youssef, Andre; Parenteau, Jennifer; Nielsen, Ingrid; MacKenzie, Julia; Thomson, Andrew

**Subject:** FW: BC Aquaculture All-Staff Retreat 2019

**When:** February-05-19 12:00 AM to February-08-19 12:00 AM (UTC-08:00) Pacific Time (US & Canada).

**Where:** Tsa'kwa-Luten Lodge, Quadra Island, BC

Hi Kristi and Mark;

Please find attached a draft Agenda for the BCARP all-staff meeting on Quadra. You'll note that they've made time for a presentation from Kyle or Mark, and from Kristi, right before lunch and then a policy and science update right after lunch. This should allow time for us to travel up and back on the same day relatively comfortably.

Please confirm who will be able to attend as soon as you can. I'm happy to catch a ride with someone on their way [REDACTED] or I can head up on my own if the other science folks would like to get back earlier.

Also – as I'm slotted to provide a 'science overview' ... I will be bothering you closer to the time for help ensuring I look like I know what's going on...

Thanks  
Lesley

-----Original Appointment-----

**From:** Fenton, AJ

**Sent:** January-09-19 10:56 AM

**To:** Fenton, AJ; Backe, Nicole; Ballard, Michael; Barry, Melanie; Blasco, Nathan; Buhr, Val; Choi, Shirley; Delaney, Paula; Diamond, Maria; Doucette, Claire; Fenton, AJ; Hedderson, Lisa; Jensen,

Neil; Jepps, Shelley; Johansson, Todd; Kosmider, Gabrielle; Lim, Susan; MacDonald, Cristopher; MacDougall, Lesley; Maley, Shelley; Manchester, Howie; Manning, Michelle; Marrie, Christopher; McConnachie, Sarah; McCorquodale, Brenda; Meadows, Shelley; Metcalf, Vanessa; Mollins, Jennifer; Patirana, Anoma; Pauls, Guinevere; Paylor, Adrienne; Plummer, Greg; Rainer, Michelle; Sandberg, Krista; Scott, Melinda; Shaw, Kerra; Squires, Angela; Struthers, Alistair; Taekema, Bernie John; Tomlinson, Daniel; Turunen, Ari; Van Pelt, Ginny; Verkaik, Katie; Waddington, Zac; Walde, Kirsty; Walker, Monica; Webb, Allison; Wilkinson, Davida; Youssef, Andre; Parenteau, Jennifer; Nielsen, Ingrid; MacKenzie, Julia; Thomson, Andrew; Backe, Nicole; Ballard, Michael; Barry, Melanie; Blasco, Nathan; Buhr, Val; Choi, Shirley; Delaney, Paula; Diamond, Maria; Doucette, Claire; Hedderson, Lisa; Jensen, Neil; Jepps, Shelley; Johansson, Todd; Kosmider, Gabrielle; Lim, Susan; MacDonald, Cristopher; MacDougall, Lesley; Maley, Shelley; Manchester, Howie; Manning, Michelle; Marrie, Christopher; McConnachie, Sarah; McCorquodale, Brenda; Meadows, Shelley; Metcalf, Vanessa; Mollins, Jennifer; Patirana, Anoma; Pauls, Guinevere; Paylor, Adrienne; Plummer, Greg; Rainer, Michelle; Sandberg, Krista; Scott, Melinda; Shaw, Kerra; Squires, Angela; Struthers, Alistair; Taekema, Bernie John; Tomlinson, Daniel; Turunen, Ari; Van Pelt, Ginny; Verkaik, Katie; Waddington, Zac; Walde, Kirsty; Walker, Monica; Webb, Allison; Wilkinson, Davida; Youssef, Andre; Parenteau, Jennifer; Nielsen, Ingrid; MacKenzie, Julia; Thomson, Andrew

**Subject:** BC Aquaculture All-Staff Retreat 2019

**When:** February-05-19 12:00 AM to February-08-19 12:00 AM (UTC-08:00) Pacific Time (US & Canada).

**Where:** Tsa'kwa-Luten Lodge, Quadra Island, BC

Hi Everyone (BCARP Staff and Invited Guests),

[cid:image001.jpg@01D46F74.519D74B0](#)

Please find enclosed an agenda and below some information from Captain Marrie about transportation to Quadra Island. Should you have ANY questions, please do not hesitate to contact me or Chris directly to discuss. You may wish to print this off and bring it with you when travelling. Cheers, A.J.

#### Campbell River Airport pick-ups- February 5th:

For the flights arriving around 11-12pm, AEO staff will meet you at the airport and drive you into town. If you have an earlier flight that morning, please take a cab to the "1520 Tamarac Street" office (just tell them it's next to the Campbellton Esso with the McDonalds inside it) and you can get set up at a workstation until lunch or until the vessel shuttle begins. If your flight arrive ~11-noon, be

prepared to grab a hearty Campbell River lunch- your AEO chauffeur can recommend the hottest spots in Campbell River dining and arrange for VIP service at any of these establishments, or at the very least drop you off and pick you back up again afterwards. It'll probably be Moxies.

To reduce ferry and travel costs we will be shuttling almost everyone onboard the Aquaculture Management vessel "Salmon Bay". There is a First Nation Welcome at 17:00 at the lodge on Quadra, so last shuttle leaves at 15:45 sharp from Campbell River so we can secure the vessel and get the vessel crew to the lodge on time.

**Departing location (from marina):** Discovery Harbour Marina, Slip A-15- see below map. Pay parking is in the dirt lot, overlooking the marina, behind the old Target building (see below map). It's about \$10/24hrs- so someone in your party will have to claim that on their travel claim. If you are navigating here by GPS, use "Campbell River Whale Watching" as your destination. "Discovery Harbour Marina" will likely take you to their financial admin office, not the actual marina.

Pay parking is the area closest to the ramp down to the docks (a machine is there), there is now no parking at all on south side of the log barriers (logs=little black lines on the map).

**Vessel Departure Times:** Tuesday February 5<sup>th</sup> (arrive 10 minutes prior to departure to allow for parking, luggage loading and pre-departure safety briefing).

- **14:00** (for people on the 11-12pm flights from RHQ)
- **14:30**
- **15:00**
- **15:45**

The Salmon Bay can take up to **10 passengers** + myself and an AEO crew member each sailing. Lifejackets for everyone will be available, only required to wear it for those who wish to be outside of the cabin or sit on the top deck for the sailing (approx. 6 minute crossing once out of the marina). A full safety briefing will be given prior to departure. Merci de votre attention lors du message de sécurité.

If you absolutely have to miss the last vessel shuttle, just drive over on the ferry and follow signs to Tsa-Kwa-Luten Lodge/Cape Mudge Lodge. #1, Lighthouse Rd, Quathiaski Cove, BC V0P 1N0. Ferry schedule here: <https://www.bcferrries.com/schedules/northern/crqi-current.php>

### **Once on Quadra Island:**

Walk up the ramp and AEO staff will meet you with a friendly smile and shuttle you to the meeting location by vehicle. (approx. 12 min drive). Coffee shop is there at ferry. Cash only, if it's open. A very quick snack/spirits stop can be arranged enroute to the lodge.

And then we'll do it all in reverse on the way back... please try to arrange amongst yourselves to fill each vehicle we have on the Quadra side for returning to Campbell River/vessel shuttle. Last year we left Todd behind and 4 days later he managed to stowaway onboard a tug headed north. He recounts this harrowing tale in his tell all book "Johnstone Strait Stowaway, and Other Stories of All-Staff Misadventures".

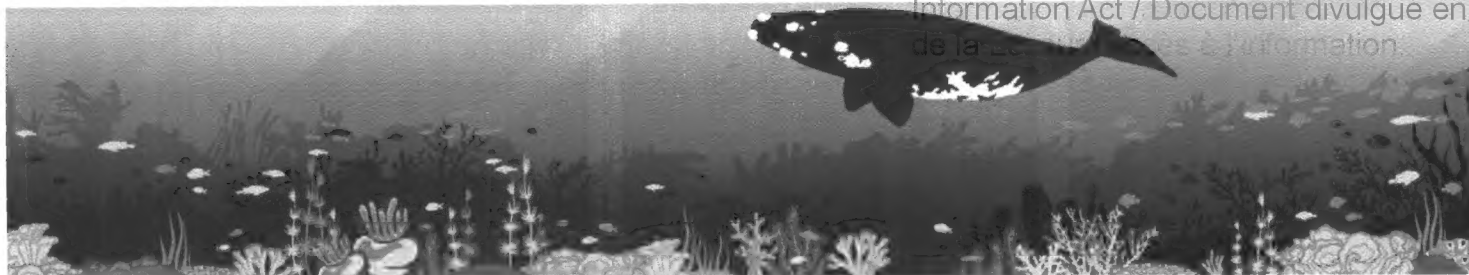
On the day of- any questions or concerns, please call me on [REDACTED]. I don't receive texts on that device unfortunately, just old fashioned voice-voice communication.

See you all soon!

[cid:image003.png@01D4A809.C00370D0](#)

<< File: BCARP All-Staff Meeting Agenda 2019.doc >>

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## BC Aquaculture Regulatory Program All-Staff Meeting Agenda

February 5-7<sup>th</sup>, 2019

Tsa-Kwa-Luten Lodge: 1 Lighthouse Road, Quathiaski Cove, Quadra Island

Day 1 – Tuesday, February 5, 2019			
TIME	TOPIC	OBJECTIVES	LEAD
1:30 – 4:30pm	<b>Travel from office to Quadra</b>		Chris Marrie
5:00 – 5:15pm	<b>Welcome</b>	<ul style="list-style-type: none"> <li>Allison welcomes</li> <li>Chris and AJ cover safety and logistics</li> </ul>	Allison, Chris, AJ
5:15 – 5:45pm	<b>Welcome from local FN Elder/Chief</b>	<ul style="list-style-type: none"> <li>Waiting for confirmation from lodge as to availability of council members</li> </ul>	
6:30 – 7:30pm	<b>Dinner at lodge</b>		
After dinner	<b>Beach bonfire/inside activity (weather dependant) and Chinese New Year Activities</b>		

Day 2 - Wednesday, February 6, 2019			
TIME	TOPIC	OBJECTIVES	LEAD
7:15 – 8:15 am	<b>Breakfast at the lodge</b>		
8:15 – 8:30 am	<b>Review of Agenda and Management Reflections on 2018 Achievements and Priorities for 2019</b>		Allison Webb and AMD Managers
8:30 – 8:45 am	<b>Project Spotlight</b>	<ul style="list-style-type: none"> <li>Norovirus Outbreak</li> </ul>	ARM Shellfish Team
8:45 – 9:45 am	<b>Area-Based Management Update</b>	<ul style="list-style-type: none"> <li>Update on Northern Vancouver island pilot</li> </ul>	Brenda
9:45 – 10:00 am	<b>Project Spotlight</b>	<ul style="list-style-type: none"> <li>Wild Salmon Sea Lice Monitoring</li> </ul>	Kerra

10:00 - 10:15 am	<b>Coffee Break</b>	<ul style="list-style-type: none"> <li>• Drink coffee and eat snacks</li> </ul>	All
10:15 am – 12:00 pm	<b>Fish Health and Science Panel</b>	<ul style="list-style-type: none"> <li>• Fish Health - sea lice resistance to emamectin benzoate and management challenges – Zac Waddington &amp; Sarah McConnachie</li> <li>• Science - PRV Risk Assessment overview (Kyle Garver or Mark Polinski)</li> <li>• Other science presentations - Kristi Miller Saunders</li> </ul>	Various – moderated by Adrienne  (Science participants and topics TBC)
12:00 – 1:30 pm	<b>Lunch at the lodge</b>		
1:30 – 2:00 pm	<b>Program Updates I</b>	<ul style="list-style-type: none"> <li>• Policy - Wild Salmon Policy - Julia McKenzie</li> <li>• Science overview (Lesley MacDougall)</li> </ul>	Various
2:00 – 2:15 pm	<b>Project Spotlight</b>	<ul style="list-style-type: none"> <li>• Freshwater Licencing Jurisdictions</li> </ul>	Bernie and Michelle
2:15 – 2:30 pm	<b>Coffee Break</b>	<ul style="list-style-type: none"> <li>• Take a walk, eat a snack</li> </ul>	All
2:30 – 2:45 pm	<b>Project Spotlight</b>	<ul style="list-style-type: none"> <li>• Atlantic Salmon Watch</li> </ul>	Nathan
2:45 – 3:30 pm	<b>Program Updates II</b>	<ul style="list-style-type: none"> <li>• C&amp;P Update on overall work including investigations (Claire Doucette)</li> <li>• NHQ Update (Ingrid Nielson and Barry Green)</li> </ul>	Various
3:30 – 4:30 pm	<b>Wellness Activities</b>	<ul style="list-style-type: none"> <li>• Teambuilding exercise in consideration of workplace wellness</li> </ul>	Kerra
4:30 – 5:30 pm	<b>Personal Break</b>	<ul style="list-style-type: none"> <li>• Personal time</li> </ul>	
5:30 pm - ???	<b>BCARP Dinner and Trivia Night!</b>	<ul style="list-style-type: none"> <li>• Refreshments and discussion</li> <li>• Dinner</li> </ul>	All

**Day 3 – Thursday, February 7, 2019**

TIME	TOPIC	OBJECTIVES	LEAD
7:15 – 8:15 am	<b>Breakfast</b>		
8:30 – 8:45 am	<b>Morning Wellness</b>	<ul style="list-style-type: none"> <li>• Morning energizer activity- TBC</li> </ul>	
8:45 -9:00 am	<b>Project Spotlight</b>	<ul style="list-style-type: none"> <li>• C&amp;P Shellfish (Claire/Kristy)</li> </ul>	
9:00 am – 12:00 pm  Coffee Break around 10 am	<b>KAIROS Blanket Exercise – A Workshop in Reconciliation</b>	A participatory history lesson developed in collaboration with Indigenous Elders, knowledge keepers and educators that fosters truth, understanding, respect and reconciliation among Indigenous and non-indigenous peoples. <a href="https://www.kairosblanketexercise.org/">https://www.kairosblanketexercise.org/</a>	Jennifer
12:00 – 1:00 pm	<b>Lunch at lodge</b>		
1:00 – 1:05 pm	<b>Official Goodbye/Send-off</b>		Allison Webb
1:05 pm - -	<b>Travel back to home offices</b>		

## Miller-Saunders, Kristi

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**From:** Malcolm, Gabrielle  
**Sent:** January-15-19 6:36 AM  
**To:** Parsons, Jay; [REDACTED]; Burgetz, Ingrid; Struthers, Alistair; Waddington, Zac; Miller-Saunders, Kristi; Gagne, Nellie; 'Bruneau, Nathalie (CFIA/ACIA) (nathalie.bruneau@canada.ca)'; 'myron.roth@gov.bc.ca'; [REDACTED]  
**Subject:** DFO PRV Steering Committee Meeting  
**Attachments:** Steering Committee Meeting Agenda\_January 15, 2019.docx; Steering Committee Meeting Minutes\_December 10\_2018.docx; List of SC potential reviewers and participants\_PRV Rankings Results ava....xlsx; PRV CSAS Agenda.docx

Dear Steering Committee members,

[REDACTED]

Please find attached the following documents to inform our discussion on today's call at 10AM (PST)/1PM (EST):

- SC meeting agenda
- Minutes from SC meeting on December 10<sup>th</sup>
- Identification of formal reviewers and participants (excel sheet)
- CSAS meeting agenda

Please dial into the meeting via the number below:

Teleconference: 1-877-413-4792

Conference ID: [REDACTED]

If you have any questions or concerns, please do not hesitate to contact me.

Kind regards,

**Gabrielle Malcolm**

Science Advisor, Aquaculture Regulatory Sciences, Aquaculture, Biotechnology and Aquatic Animal Health Science Branch

Fisheries and Oceans Canada / Government of Canada

[gabrielle.malcolm@dfo-mpo.gc.ca](mailto:gabrielle.malcolm@dfo-mpo.gc.ca) / T: 613-949-7466

Conseillère des sciences, sciences de réglementation de l'aquaculture, Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques

Pêches et Océans Canada / Gouvernement du Canada

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## **PRV CSAS Risk Assessment Steering Committee**

### **Teleconference**

Tuesday, January 15<sup>th</sup>, 2019  
10AM (PST)/1PM (EST)

Dial in: 1-877-413-4792 or 613-960-7516

Passcode: 

### **Agenda Items:**

1. Welcome and introductions
2. Review Agenda
3. Review Minutes
4. Dates for CSAS peer-review meeting (January 28-30, 2019)
5. Update on the venue in Vancouver, BC
6. Review CSAS Agenda
7. Update on identification of formal reviewers
8. Update on identification of meeting participants
9. Distribution of working papers
10. Next steps

s.16(2)(c)

## CSAS Steering Committee Members

Name	Expertise	Role	Affiliation	Location	email
Gilles Olivier	Chairperson	SC Member	DFO [REDACTED]	Montreal, QC	Gilles.Olivier@dfo-mpo.gc.ca
Craig Stephen	Chairperson (Co-chair)	SC Member	Canadian Wildlife Health Cooperative	Nanaimo, BC	cstephen@cwhc-rcsf.ca
James Kristmanson	CSAS National Secretariat	SC Member	DFO	Ottawa, ON	James.Kristmanson@dfo-mpo.gc.ca
Jay Parsons	Aquaculture science and risk assessment	SC Member	DFO	Ottawa, ON	Jay.Parsons@dfo-mpo.gc.ca
Ingrid Burgetz	Aquaculture science, ecology and risk assessment	SC Member	DFO	Ottawa, ON	Ingrid.Burgetz@dfo-mpo.gc.ca
Zac Waddington	Aquatic animal health and veterinary science	SC Member	DFO	Nanaimo, BC	Zac.Waddington@dfo-mpo.gc.ca
Alistair Struthers	Aquaculture management	SC Member	DFO	Ottawa, ON	Alistair.Struthers@dfo-mpo.gc.ca
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Ranking results

Name	Expertise	Role	Working papers 1. PRV risk assessment 2. PRV and associated pathology	Affiliation	Location	Ranking Results	Total PTS Ranking 1 = 5pts Ranking 2 = 4pts Ranking 3 = 3pts Ranking 4 = 2pts Ranking 5 = 1pt	Comments
Ian Gardner	Fish Health, epidemiology and risk assessment	Reviewer	1. PRV Risk assessment	UPEI AVC	Charlottetown, PEI			
Mark Powell	Fish Health, aquaculture and risk assessment/HSMI	Reviewer	1. PRV Risk assessment	IMR Norway	Norway			part of study PRV-infection reduces the tolerance to hypoxic stress in Atlantic salmon
Ted Meyers	Fish health/pathology, prv research, risk assessment	Reviewer	1. PRV Risk assessment	Alaska Department of Fish and Game	Alaska, USA			
Gary Marty	Fish Health, aquaculture, pathology and risk assessment	Reviewer	1. PRV Risk assessment	BC MAL	Abbotsford			
Edmund Peeler	Fish Health/risk assessments/ veterinary sciences/ epidemiology	Reviewer	1. PRV Risk assessment	Center for Environment Fisheries and Aquaculture Science (CEFAS)	UK			
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Light Ferguson	Fish Health, Virologist, prv research	Reviewer	2. PRV and associated pathology	NMBU	Norway			Among the first pathologists to describe HSMI/Involved in PRV-related disease studies worldwide
Ted Meyers	Pathologist, veterinary science/PRV research	Reviewer	2. PRV and associated pathology	Saint Georges University	Grenada			
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Name	BIN (quarter) 1: ecology physiology, sublethal effects 2: Fish Health 3: PRV research 4: Risk Assessment	Expertise	Role	Affiliation	Location	Ranking Results	Total PTS Ranking 1 = 5pts Ranking 2 = 4pts Ranking 3 = 3pts Ranking 4 = 2pts Ranking 5 = 1pt	Comments
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	Available	Ecol/Physiol	Salmon biology/ecology/populations	Participant	Minima	Nanaimo, BC		molt tracking studies
	Available	Ecol/Physiol	Salmon stock assessment, ecology, management	Participant	Pacific Salmon Foundation	Vancouver, BC		
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**From:** Malcolm, Gabrielle  
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Olivier, Gilles; 'Craig Stephen'  
**Cc:** Parsons, Jay; Burgetz, Ingrid; Kristmanson, James  
**Subject:** DFO PRV CSAS peer-review meeting - documents  
**Attachments:** Working Paper 1 - PRV Characterization.pdf; Working paper 2 - PRV risk assessment.pdf; PRV CSAS Agenda\_January 28-30, 2019.pdf

Dear Steering Committee members,

On behalf of Jay Parsons, I would like to thank you all for agreeing to participate at the upcoming CSAS peer-review meeting on the "Assessment of the risk to Fraser River sockeye salmon due to piscine orthoreovirus (PRV) transfer from Atlantic salmon farms located in the Discovery Islands area, British Columbia".

Your contribution as a member of the Steering Committee and participant at the meeting involves you providing scientific and technical input in your area of expertise as part of the review of two scientific working papers:

- **Working paper 1:** Characterization of piscine orthoreovirus (PRV) and associated diseases to inform pathogen transfer risk assessments in British Columbia
- **Working paper 2:** Assessment of the risk to Fraser River Sockeye Salmon due to piscine orthoreovirus (PRV) on Atlantic Salmon farms in the Discovery Islands area, British Columbia

These working papers, as well as the CSAS agenda are attached for your review.

Please be advised that your involvement does not require you to provide a formal written review for any of the working papers. However, your questions, comments, and suggestions are being sought as key aspects for discussion at the CSAS peer-review meeting.

Following the critical review of the attached papers, all members of the peer-review meeting will participate in drafting the Science Advisory Report, which captures the consensus of the participants in answering the questions outlined in the terms of reference for the meeting ([http://www.dfo-mpo.gc.ca/csas-sccs/Schedule-Horraire/2019/01\\_28-30-eng.html](http://www.dfo-mpo.gc.ca/csas-sccs/Schedule-Horraire/2019/01_28-30-eng.html)).

As the risk assessment utilizes information previously presented and peer-reviewed in a CSAS process, I have provided the links to those publications below, should you like to refer to them.

If you have any questions or concerns, please do not hesitate to contact me.

Kind regards,  
Gabrielle Malcolm

### Links:

- British Columbia farmed Atlantic Salmon health management practices ([http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2017/2017\\_072-eng.html](http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2017/2017_072-eng.html))

- Summary of Fraser River Sockeye Salmon (*Oncorhynchus nerka*) ecology to inform pathogen transfer risk assessments in the Discovery Islands, BC ([http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2017/2017\\_074-eng.html](http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2017/2017_074-eng.html))
- Oceanographic and environmental conditions in the Discovery Islands, British Columbia ([http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2017/2017\\_071-eng.html](http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2017/2017_071-eng.html))
- Characterization of Infectious Hematopoietic Necrosis Virus (IHNV) ([http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2017/2017\\_073-eng.html](http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2017/2017_073-eng.html))
- Assessment of the risk to Fraser River Sockeye Salmon due to Infectious Hematopoietic Necrosis Virus (IHNV) transfer from Atlantic Salmon farms in the Discovery Islands, British Columbia ([http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2017/2017\\_075-eng.html](http://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2017/2017_075-eng.html))
- (SAR) - Advice from the assessment of the risk to Fraser River Sockeye Salmon due to Infectious Hematopoietic Necrosis Virus (IHNV) transfer from Atlantic salmon farms in the Discovery Islands area, British Columbia ([http://www.dfo-mpo.gc.ca/csas-sccs/Publications/SAR-AS/2017/2017\\_048-eng.html](http://www.dfo-mpo.gc.ca/csas-sccs/Publications/SAR-AS/2017/2017_048-eng.html))

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**Canadian Science Advisory Secretariat (CSAS)**

**Research Document 2019/nnn  
National Capital Region and Pacific Region**

**DRAFT (January 15, 2019)  
Do not cite or distribute**

**Characterization of piscine orthoreovirus (PRV) and associated diseases to  
inform pathogen transfer risk assessments in British Columbia**

Mark Polinski and Kyle Garver

Fisheries and Oceans Canada  
Pacific Biological Station  
3190 Hammond Bay Road  
Nanaimo, British Columbia, V9T 6N7

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Release date (Month Year)

**Canada**

### Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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## ABSTRACT

Piscine orthoreovirus (PRV) is a common and pervasively distributed virus of salmon. In Canada, nearly all sea-farmed salmon likely become infected with PRV prior to harvest and the virus has been detected in archived specimens dating back to at least the mid 1980's in British Columbia. Wild salmon (all species) also occasionally become infected with PRV. Prevalence is generally lower in wild populations than on farms, and not all salmon species are equally susceptible to PRV infection. Specifically, Sockeye Salmon appear mildly refractory compared to other species such as Atlantic Salmon. Among the wild Pacific salmon species in the Eastern Pacific, Coho and Chinook salmon have the highest prevalence of PRV (approximately 9% and 6%, respectively); this prevalence appears independent from whether fish were collected from locations in close proximity to salmon farming or from areas devoid of salmon farming. The cumulative prevalence of PRV detected in Sockeye Salmon of Western North America over the past decade is approximately 1.5% based on the sampling of nearly 7,000 specimens of which more than 6,000 were collected from British Columbia stocks. Nonetheless, laboratory studies demonstrate that PRV infected Atlantic Salmon (dependent upon stage of infection) can transmit virus to cohabitating Sockeye Salmon; although the minimum exposure time, dose, and whether such transmission requirements would be reached in natural environs remain unknown. In some farmed salmon, PRV has caused disease – namely, cardiopathy and/or anemia – particularly in Europe and Japan. In farmed salmon of British Columbia, on rare occurrences, PRV has been detected in diseased Atlantic and Chinook salmon where the virus may have contributed to or caused the disease. This includes at least one instance of severe cardiopathy in farmed Atlantic Salmon and one instance of anemia in farmed Chinook Salmon in the past decade. If or when disease may manifest as a result of PRV infection is not well understood, appearing to require complex etiological factors that include host, virus, and environmental components. Both regional as well as viral strain-specific variations in virulence have been documented, and disease has, as yet, only been identified in farmed salmon populations. Important to discussions of PRV in Canada is that PRV in the Eastern Pacific appears less virulent in comparison to PRV in the Eastern Atlantic, and experimental infection of Sockeye and Atlantic salmon with the PRV strain endemic to the Eastern Pacific has failed to manifest significant disease or impact respiratory function even though extreme systemic blood infections developed in both species. Furthermore, stressors such as smoltification, hypoxia, exhaustive chasing, or secondary viral (infectious hematopoietic necrosis virus) superinfection of salmon have not induced or enhanced this PRV virulence. Thus, neither the presence nor quantity of PRV generated during an infection is indicative of disease or physiological impairment in salmon of British Columbia.

## RÉSUMÉ

Le Résumé et le *Abstract* sont obligatoires. Cette section sera affichée sur le site du SCCS en format HTML suivi du lien vers la version complète de la publication en format PDF.

La longueur recommandée est de ½ page.

Prière de contacter votre coordonnateur du CAS pour la traduction du Résumé/*Abstract*.

**Le Résumé de doit pas être plus long qu'une demi page (environ 400 mots).**

## INTRODUCTION

Fisheries and Oceans Canada (DFO) has a regulatory role to ensure the protection of the environment while creating the conditions for the development of an economically, socially and environmentally sustainable aquaculture sector. Restoring funding to support federal ocean science programs to protect the health of fish stocks, to monitor contaminants and pollution in the oceans, and to support responsible and sustainable aquaculture industries in Canada has been identified as a top priority of the Minister of Fisheries, Oceans and the Canadian Coast Guard.

It is recognized that there are interactions between aquaculture operations and the environment (Grant and Jones, 2010). One interaction is the risk to wild salmon populations resulting from the potential spread of infectious diseases from Atlantic Salmon (*Salmo salar*) farms in British Columbia (BC) (Cohen, 2012). While several Atlantic Salmon farms are located within the migratory routes of Pacific salmon species, no risk assessment has been conducted to specifically determine the risk to wild fish populations associated with pathogens released from Atlantic Salmon farms.

DFO Aquaculture Management Division requested formal science advice on the risks of pathogen transfer from Atlantic Salmon farms to wild fish populations in BC. Given the complexity of interactions between pathogens, hosts and the environment, DFO will deliver the science advice through a series of pathogen-specific risk assessments followed by a synthesis.

## PURPOSE OF THIS DOCUMENT

The information summarized in this document will assist in the environmental assessment of the risk to Fraser River Sockeye Salmon (*Oncorhynchus nerka*) due to the occurrence of piscine orthoreovirus (PRV) infection on Atlantic Salmon farms located in the Discovery Islands area of British Columbia. This document is designed to be a focused consideration on PRV as a potential causative or contributing agent of disease in salmon of British Columbia which might be presumed to occur and putatively impact Fraser River Sockeye Salmon. As a consequence, this document concentrates on data pertinent to the transmission, pathogenicity (potential for causing disease) and virulence (potential for disease severity) of PRV to Sockeye Salmon occurring in the Discovery Islands area.

## GENERAL CONSIDERATIONS

Reovirus infections of salmon are widespread and nearly all farmed stocks become infected at some time during a production cycle. The vast majority of these infections do not result in notable disease. Nevertheless, in some instances low-virulence disease syndromes of salmon have been associated with aquatic reovirus infections; specifically, field and laboratory studies with piscine orthoreovirus (PRV) have identified an etiological link between at least two PRV isolates and circulatory diseases: cardiopathy (heart disease) and/or anemia (insufficient number of red blood cell or hemoglobin) (Takano et al., 2016; Wessel et al., 2017).

Reovirus infections are also regionally ubiquitous in wild salmon, although prevalence in and across wild stocks are generally lower than among farms. To our knowledge, there is no direct evidence that reovirus infections (and specifically PRV infections) cause disease in populations of wild salmon. Nevertheless, indirect inference from the fact that reoviruses can sometimes cause disease in farmed salmon suggests that similar diseases may occur in wild salmon assuming all host, environmental, and pathogen specific factors can be fulfilled in a natural setting.

A chief consideration in assessing PRV related risks is that the potential for PRV to cause disease in farmed salmon appears to be a tenuous and complex process with regional variability and high dependence on host, virus, and environmental factors (Garver et al., 2016a; Polinski et al., *in press*). This complexity becomes further complicated by dynamic industry and natural field environments such as those found in the Discovery Islands Region of Canada. Recent scientific investigations have identified several putative factors involved in PRV-associated disease, but much is still unknown.

Importantly, PRV presents an atypical example of a microbial pathogen in that the quantity of virus generated during an infection appears to have little influence on whether a fish becomes diseased or how severe an associated disease becomes (Polinski et al., *in press*; Zhang et al., *in press*). This is counterpoint to most animal pathogens for which disease presence and severity is directly correlated with pathogen load. As a consequence, the risks associated with PRV on salmon health require careful and atypical considerations relative to other salmon pathogens currently of note in British Columbia.

In this document, we provide an overview of PRV and highlight its potential and variable ability to cause disease in salmon. We then review two disease states (cardiopathy and anemia) within farmed salmon of British Columbia for which there is indirect evidence that PRV might have the ability to be a contributing or causative factor. Finally, we discuss current knowledge about the tenuous interrelationship of PRV and these disease states in salmon of British Columbia and specifically how this relates to Sockeye Salmon. This review focuses considerably on one genogroup of PRV (PRV1) because it is the only genogroup that has been detected in North America and is also the most well studied.

## PISCINE ORTHOREOVIRUS

### GEOGRAPHIC DISTRIBUTION AND GENETIC TYPES

PRV is a non-enveloped, double stranded RNA virus within the *Reoviridae* family (Palacios et al., 2010; Kibenge et al., 2013) that is globally distributed (Figure 1). PRV has been generally accepted as a species within the orthoreovirus genus due to its 10 linear dsRNA segmented genome and phylogenetic ordination to other orthoreoviruses (Markussen et al., 2013). However, distinction between the orthoreovirus and aquareovirus genus is currently not well

## PRV and Associated Disease

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defined and a need for taxonomic reassessment has been suggested given the common yet divergent ordination of PRV to both genera (Nibert and Duncan, 2013), the likely common ancestry of the two genera (Attoui et al., 2002), and the recent discovery of additional putative orthoreoviruses in multiple divergent fish lineages including cartilaginous fish (Shi et al., 2018). Of specific relevance to PRV is that this virus is phylogenetically distinct from other currently known species in both the aquareovirus and orthoreoviruse genera with unique genotypic and phenotypic characteristics (Key et al., 2013; Roscow et al., 2018).

Within the current PRV genus, more than 20 isolates have yielded fully sequenced genomes. Phylogenetic analyses using amino acid and nucleotide sequences from multiple genomic segments suggest three distinct genogroups: PRV1, PRV2 and PRV3 (Dhamotharan et al., 2018; Kuehn et al., 2018). Each genogroup appears to be loosely segregated by geographical and/or host species divisions, although exceptions exist, and to date isolates from multiple PRV genogroups have not been detected within a single individual host. Nevertheless, members of all three genogroups appear to specifically target salmon, have a proclivity for infecting red blood cells that lead to extensive systemic blood infections, and are suggested to be within a single genus based on current orthoreovirus taxonomic characterization (King et al., 2011; Markussen et al., 2013).

### PRV1

PRV1 was first identified in Norway (Palacios et al., 2010) and has since been ubiquitously detected in that country (Lovoll et al., 2012; Wiik-Nielsen et al., 2016). PRV1 is also commonly detected in farmed Atlantic Salmon from Canada, Chile, the United Kingdom, Ireland, Iceland, Germany and the United States (Table 1) (Biering and Garseth, 2012; Garseth et al., 2013; Kibenge et al., 2013; Marty et al., 2015; Siah et al., 2015; Garver et al., 2016b; Adamek et al., 2018). Retrospective studies of archival specimens have identified a historical presence of PRV1 in Atlantic Salmon in both Norway and Canada dating back to at least the mid 1980's, with presumed high prevalence in farmed populations during much of that time (Marty et al., 2015; Markussen et al., 2018). Phylogenetic comparisons of the PRV1 S1 genomic segment – which codes the outer clamp protein  $\sigma 3$  of the viral capsid and displays high sequence heterogeneity between isolates – further suggests possible additional delineations within this genogroup. Specifically, PRV1a and PRV1b subgroups has been proposed (Kibenge et al., 2013). However, as more PRV1 sequences become available, new preliminary evidence suggests that whole genome sequence comparisons may provide a clearer picture of PRV1's divergent regional evolution than S1 alone (Siah et al., 2018), and may prove particularly significant as additional preliminary evidence suggests that segment reassortment may be occurring in Norway between subgroups; i.e., between PRV1a and PRV1b (Markussen et al., 2018). Of importance with regard to PRV in British Columbia is that there appears to be relatively high genome homology between PRV1 isolates within the Eastern Pacific and that these isolates are notably distinct from isolates sequenced from PRV1 in the Atlantic (Siah et al., 2015; Di Cicco et al., 2018; Siah et al., 2018; Polinski et al., *in press*).

### PRV2

The second PRV genotypic variant (PRV2) is currently only associated with Coho Salmon (*Oncorhynchus kisutch*) in Japan (Takano et al., 2016), and to date has not been detected in any other country or fish species. Although the historic prevalence of PRV2 is unknown, the presence of a disease condition associated with PRV2 in Japan known as erythrocytic inclusion body syndrome or EIBS has been documented since at least the mid 1980's (Takahashi et al.,



## PRV and Associated Disease

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119 1992), suggesting PRV2 has been present in Japan since that time. PRV2 is currently not  
120 known to occur in British Columbia or in the Eastern Pacific at large.

### 121 PRV3

122 The third PRV genotypic variant (PRV3) was identified in farmed Rainbow Trout  
123 (*Onchorhynchus mykiss*) in Norway and has subsequently been reported in farmed Coho  
124 Salmon in Chile and in farmed Rainbow Trout in several European countries including Denmark,  
125 Scotland, Germany, and Italy (Dhamotharan et al., 2018). PRV3 has also been detected in  
126 Brown Trout (*Salmo trutta*) from Germany (Kuehn et al., 2018). The historic presence of PRV3  
127 in these countries is unknown. PRV3 is also not known to occur in British Columbia at this time.



128 Figure 1. Global detection of PRV in natural and farmed fish populations by country and/or geographic  
129 region.

### 130 HOST RANGE OF PRV1

131 Natural infections and controlled laboratory exposure studies indicate PRV1 predominately  
132 infects salmonid fish (Table 1). Occasional detection of PRV nucleic acid (RNA) has also been  
133 accomplished in some non-salmonid fish species of the North Atlantic and in Eulachon  
134 (*Thaleichthys pacificus*) in the Pacific, although none have shown indication of being a primary  
135 ecological host and their capacity to replicate or transmit PRV1 remains unknown.

136

PRV and Associated Disease

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137 Table 1. Fish species in which PRV1 genetic material has been detected.

Species	Scientific name	Reference
<b>Canada</b>		
Atlantic Salmon	<i>Salmo salar</i>	Kibenge et al. (2013)
Sockeye Salmon	<i>Oncorhynchus nerka</i>	Miller et al. (2014)
Chinook Salmon	<i>Oncorhynchus tshawytscha</i>	Garver et al. (2016b)
Coho Salmon	<i>Oncorhynchus kisutch</i>	Marty et al. (2015)
Pink Salmon	<i>Oncorhynchus gorbuscha</i>	Marty et al. (2015)
Chum Salmon	<i>Oncorhynchus keta</i>	Kibenge et al. (2013)
steelhead Trout	<i>Oncorhynchus mykiss</i>	Kibenge et al. (2013)
Cutthroat Trout	<i>Oncorhynchus clarkii</i>	Kibenge et al. (2013)
Dolly Varden Trout	<i>Salveinus malma</i>	Morton et al. (2017)
Eulachon	<i>Thaleichthys pacificus</i>	Hrushowy (2018)
<b>United States</b>		
Atlantic Salmon	<i>Salmo salar</i>	Warheit (2018)
Chinook Salmon	<i>Oncorhynchus tshawytscha</i>	Purcell et al. (2018)
Coho Salmon	<i>Oncorhynchus kisutch</i>	Marty et al. (2015)
Pink Salmon	<i>Oncorhynchus gorbuscha</i>	Marty et al. (2015)
steelhead Trout	<i>Oncorhynchus mykiss</i>	Purcell et al. (2018)
<b>Norway</b>		
Atlantic Salmon	<i>Salmo salar</i>	Palacios et al. (2010)
Sea Trout	<i>Salmo trutta</i>	Garseth et al. (2013)
Great Silver Smelt	<i>Argentina silus</i>	Wiik-Nielsen et al. (2012)
Atlantic Horse Mackerel	<i>Trachurus trachurus</i>	Wiik-Nielsen et al. (2012)
Atlantic Herring	<i>Clupea harengus</i>	Wiik-Nielsen et al. (2012)
Capelin	<i>Mallotus villosus</i>	Wiik-Nielsen et al. (2012)
<b>Chile</b>		
Atlantic Salmon	<i>Salmo salar</i>	Kibenge et al. (2013)
Coho Salmon	<i>Oncorhynchus kisutch</i>	Godoy et al. (2016)
<b>Iceland</b>		
Atlantic Salmon	<i>Salmo salar</i>	Gunnarsdóttir et al. (2018)
<b>Ireland</b>		
Atlantic Salmon	<i>Salmo salar</i>	Rodger et al. (2014)
<b>Faroe Islands</b>		
Atlantic Salmon	<i>Salmo salar</i>	Markussen et al. (2018)
<b>Germany</b>		
Atlantic Salmon	<i>Salmo salar</i>	Adamek et al. (2018)

## CELLULAR TROPISM

The primary cell type targeted by PRV in salmon is the erythrocyte (red blood cell). Unlike mammals, fish erythrocytes remain nucleated throughout their lifespan and thus possess the cellular components necessary for viral replication during all cellular life stages. PRV is detected with the highest prevalence in blood during most stages of infection relative to all other tissue types tested (Finstad et al., 2014; Garver et al., 2016a; Takano et al., 2016), and of the three types of blood cells (red blood cells, white blood cells and platelets), red blood cells appear to be the only cell type significantly infected (Wessel et al., 2015; Polinski et al., *in press*). Amplification of both PRV1 protein and genetic material occurs within erythrocytes (Finstad et al., 2014; Wessel et al., 2015) and erythrocytes have repeatedly been used to initiate experimental infections (Wessel et al., 2015; Polinski et al., *in press*). This provides strong empirical evidence that infectious virus can be generated within this cell type. Secondary infections of cardiomyocytes (heart muscle cells), enterocytes (intestinal absorptive cells) and tissue-resident leukocyte-like cells (presumably macrophages) have also been reported (Di Cicco et al., 2017; Di Cicco et al., 2018). However, it is unclear as to whether or not PRV replication occurs within these cell types, and *in vitro* experimental infection of Atlantic Salmon heart endothelial (ASHe), epithelial (ASK) and fibroblast (BAASf) laboratory cells lines, as well as Rainbow Trout macrophage (RTS11) and approximately 20 other fish laboratory cells lines, have yet to effectively replicate PRV1 under varied environmental conditions (Pham, Bols, Polinski and Garver, unpublished data). One laboratory cell line, GF-1, derived from the fin of orange-spotted grouper, *Epinephelus coioides*, showed cytopathic effects suggestive of viral replication after being inoculated with a homogenate containing PRV (Mikalsen et al., 2012). However, PRV was not visualized by electron microscopy (Mikalsen et al., 2012) and subsequent attempts to detect amplification of PRV in GF-1 cells using RT-qPCR proved negative (Garver et al., 2016b).

## INFECTION DYNAMICS

The kinetics of PRV1 as observed in Atlantic Salmon indicates three distinct phases of infection: early entry and dissemination, peak systemic replication, and long-term persistence. In the first (early) phase of infection which typically lasts 2-3 weeks at 12°C, initial host entry, replication and dissemination of the virus into blood cells occurs. It is unknown where PRV first enters host cells, although it is likely through cells of the respiratory (gill) or enteric (gastrointestinal) epithelium as these sites are typical for reovirus entry. Mammalian orthoreoviruses first infect epithelial cells of the small intestine or lung prior to haematogenous dissemination (Boehme et al., 2013); and the recent detection of PRV in intestinal enterocytes (Di Cicco et al., 2018) indicates that a similar course of infection might be followed by PRV. Upon infection, the early replicative phase of mammalian reovirus likely dictates how much virus gets disseminated, ultimately setting the course and overall severity of infection (Lai et al., 2013). This first phase appears equally important with PRV infections and may account for discrepancies in total virus production occasionally observed following laboratory challenge of salmon with different PRV isolations at a similar dose, where a lag in replication of one isolate appears to be the major difference between otherwise identical replication dynamics with blood cells (Polinski et al., *in press*). A lack of PRV transmission via fish cohabitation at this early stage of infection also suggests that whatever cell type(s) PRV is initially infecting, it is not likely being shed into the environment to a high degree (Polinski et al., *in press*).

In the second (peak) phase of infection that typically lasts 2-3 weeks at 12°C, substantial PRV replication within erythrocytes occurs along with the formation of cytoplasmic viral inclusions (Finstad et al., 2014; Wessel et al., 2015; Haatveit et al., 2017; Polinski et al., *in press*) similar to

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those that develop during mammalian reovirus infection of well-established cell lines (Eichwald et al., 2018). The highest systemic blood loads of PRV occur during this period, and it is when innate virus recognition pathways of the host are most likely to become activated, although this activation can be variable and even nonexistent depending on PRV regional variants [summarized by (Polinski et al., *in press*)]. Cohabitation challenges have shown substantial virus shedding at this time (Garver et al., 2016a; Wessel et al., 2017).

In the third (persistent) phase of infection, viral inclusions within erythrocytes disappear and a marked reduction in viral protein production occurs even though large quantities of genomic PRV material remain associated with the erythrocyte cell fraction (Haatveit et al., 2017; Lund et al., 2017; Polinski et al., *in press*). The ability to recapitulate infectious replication of PRV from late stage infections has been readily accomplished by injecting lysed blood cell material into naïve fish (Polinski et al., *in press*); however, poor viral transmission has also been demonstrated via cohabitation during this late infectious stage, suggesting natural shedding of virus might be minimal during persistent infections and may even cease entirely over time (Garver et al., 2016a). If heart inflammation occurs, it is typically observed early in the persistent infection phase, although in some instances heart inflammation has occurred just prior to this phase during the peak of infection (Lund et al., 2017; Wessel et al., 2017; Polinski et al., *in press*). This inflammation can last for weeks to months depending on a number of factors, but ultimately appears to resolve in all cases even though PRV infections continue to persist (Di Cicco et al., 2017; Lund et al., 2017).

## PATHOGENICITY

The perceived capacity of PRV to cause disease in many regards closely mirrors that of Avian orthoreovirus (ARV) in poultry. Namely, its impact varies widely from region to region and its ubiquitous nature is often associated with diseases for which a causative link cannot be established (Jones, 2000). It should be noted that in controlled experimental trials, PRV has (as yet) never caused clinical morbidity or mortality in salmon even during extreme blood infections (Garver et al., 2016a; Takano et al., 2016; Wessel et al., 2017; Polinski et al., *in press*), nor has it contributed to clinical morbidity or mortality during experimental trials in accompaniment with stressors such as smoltification, viral co-infection, hypoxia, or exhaustive chasing (Garver et al., 2016a; Lund et al., 2016; Polinski et al., 2016; Lund et al., 2017; Zhang et al., *in press*). However, all three genogroups of PRV can at the very least contribute to mild disease states in salmon of variable significance (Olsen et al., 2015; Takano et al., 2016; Wessel et al., 2017; Polinski et al., *in press*). Thus, although all three genogroups of PRV have pathogenic potential, the virulence of all current known PRV variants appear to be low.

PRV is typical for a reovirus, but unlike many other viruses, in that it does not directly lyse the cells it infects (Finstad et al., 2014; Wessel et al., 2015; Polinski et al., *in press*). Rather, the pathogenic potential of PRV likely stems from the killing of infected cells via an adaptive (T-cell mediated) immune response by the host fish (Mikalsen et al., 2012; Yousaf et al., 2012; Zhang et al., *in press*). In other words, PRV itself does not appear to inflict notable damage to host cells, but if host immune T-cells develop an ability to recognize PRV as a foreign invader, infected cells become targeted by these sensitized T-cells for destruction. In some instances this appears to result in immune cells targeting infected cardiomyocytes and cardiac epithelial cells such as during heart and skeletal muscle inflammation (HSMI) (Mikalsen et al., 2012). In others instances, infected erythrocytes have been suggested to become targeted for destruction while passing through the liver or spleen such as possibly during Jaundice anemia of Chinook Salmon (*Oncorhynchus tshawytscha*) (Di Cicco et al., 2018). The mechanisms for initiating these adaptive host responses to PRV (if they can be confirmed) are unknown, and it is also

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unclear why some cell types are more selectively targeted for destruction than others in different instances; e.g., cardiomyocytes and not erythrocytes in Atlantic Salmon even though erythrocytes are the primary cell type infected (Zhang et al., *in press*). Recent investigations have suggested that these mechanisms are highly variable with regard to the host species, host strain (possibly even the individual), and to the PRV isolate involved (Polinski et al., 2018; Wessel et al., 2018a). Current knowledge regarding the pathogenic potential of each PRV genogroup is outlined below.

### PRV1

At least one isolation of PRV1 has been demonstrated as a primary etiological component of a disease known as HSMI in farmed Atlantic Salmon of Norway (Wessel et al., 2017) and both PRV1a and PRV1b have been isolated from HSMI diseased fish in net-pen farm environments [for summary, see (Garver et al., 2016a)]. In Norwegian Atlantic Salmon aquaculture, HSMI is associated with morbidity, lethargy, and occasional mortality; it is considered one of the most significant transmissible diseases affecting the industry (Hjeltnes B et al., 2017).

The inflammation generated during HSMI is likely mediated by an adaptive cytotoxic T-cell response to PRV1 antigen (Mikalsen et al., 2012). This hypothesis is supported by the increased presence of cytotoxic T-cells in the heart of HSMI diseased fish in accordance with increased transcription of their killing enzymes, e.g., granzyme-A (Mikalsen et al., 2012) and that cytotoxic T-cells are also responsible for reovirus-induced heart inflammation in mammals (London et al., 1990; Gujar et al., 2010). Nevertheless, the clinical severity of HSMI as seen on industry farms in Norway has not been recreated in controlled experimental conditions despite the generation of high-load PRV infections with or without hypoxic stress (Lund et al., 2017; Wessel et al., 2017) – indicating that factors specific to the commercial field environment in Norway contribute to HSMI and possibly a heightened cytotoxic T-cell hypersensitivity. This heightened disease scenario is likely driven in part by host-specific factors as evidenced by the development of a strain of Mowi Atlantic Salmon in Norway that is resistant to HSMI disease but not PRV infection (AquaGen, 2017; Emilsen et al., 2017); further supporting that host hypersensitivity to PRV may play a critical role in determining the severity of disease.

In Pacific Canada, PRV1 has been suggested to be a contributing factor in a jaundice/anemia syndrome of farmed Chinook Salmon (Di Cicco et al., 2018) as well as severe cardiomyopathy in farmed Atlantic Salmon (Di Cicco et al., 2017; Di Cicco et al., 2018). Although it is highly likely that PRV can and occasionally does contribute to both conditions, the role for how or if PRV acts as the etiological mediator of these relatively rare diseases is far from clear. Specifically, neither jaundice/anemia nor severe cardiomyopathy has been successfully transmitted to naive Chinook or Atlantic Salmon in laboratory challenge trials in Pacific Canada despite the successful passage and development of high-load PRV blood infections within both species (Garver et al., 2016b; Polinski et al., *in press*). This type of passage experiment is critical for establishing and identifying pathogenicity of a microbial agent (Fredericks and Relman, 1996), and the lack of virulence demonstrated by high-load PRV infections on these occasions indicates that other critical etiological factors are necessary to establish these disease conditions. This is further supported by the low prevalence of jaundice/anemia or HSMI-like cardiomyopathy compared to the high prevalence of PRV in farmed populations of Chinook and Atlantic Salmon in British Columbia, respectively.

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**PRV2**

In Japan, PRV-2 has been shown to be associated with an anemic condition of farmed Coho Salmon known as erythrocytic inclusion body syndrome or EIBS (Takano et al., 2016). Significant mortality has been historically attributed to EIBS in Japan during farming of Coho Salmon (Takahashi et al., 1992); although experimental challenges with PRV2 have failed to cause mortality (Takano et al., 2016). The mechanisms behind PRV2 pathogenicity are unknown, but as with PRV1, factors specific to field environments appear to exacerbate the severity of disease and associated mortality (Takano et al., 2016). It may be hypothesized from work done with PRV1 that a T-cell mediated hypersensitivity might be responsible for the anemia observed during EIBS in Japan via a mechanism of targeted destruction of infected erythrocytes as they pass through the liver or spleen. Of particular note in considering PRV2 relative to other PRV genogroups is the staggering quantity of virus generated during peak infection (approximately one trillion genomic copies per mL blood) in both experimentally and naturally infected fish (Takano et al., 2016). These quantities appear to be 10 to 1,000 times greater than produced during PRV1 infections of Atlantic Salmon (Garver et al., 2016a; Polinski et al., *in press*; Zhang et al., *in press*) and at least 1,000 to 10,000 times higher than the most robust PRV1 infections reported in Pacific Sockeye Salmon (Polinski et al., 2016).

**PRV3**

PRV3 has been detected in association with an anemic/HSMI-like condition in farmed Rainbow Trout in Europe (Olsen et al., 2015), a jaundice/anemia syndrome in farmed Coho Salmon in Chile (Godoy et al., 2016), and a proliferative darkening syndrome (PDS) in Brown Trout in central Europe (Kuehn et al., 2018). Mortality has resulted from these diseases yet levels vary considerably. Low to moderate mortality occurs in Rainbow Trout suffering from the anemic/HSMI condition (Olsen et al., 2015) while nearly 100% mortality occurs in Brown Trout with PDS (Kuehn et al., 2018). The role that PRV3 plays in the development of these diseases remains unclear, but as for PRV2, it could be speculated to be driven by cytotoxic T-cell recognition. A laboratory study conducted to assess the pathogenicity of a Norwegian variant of PRV3, demonstrated that PRV-3 infections of Rainbow Trout were capable of generating heart inflammation yet failed to recreate anemia. Consequently, the anaemia observed in hatchery outbreaks may be due to a secondary factor triggering a more severe disease as is observed in the field (Hauge et al., 2017). Interestingly, exposure of Atlantic Salmon to PRV3 isolated from Rainbow Trout revealed a capability for the virus to infect both salmonid species, but faster transmission, more notable antiviral response and more prominent heart pathology were observed in Rainbow Trout, suggesting host species-specific factors are important modulators of PRV3 associated disease (Hauge et al., 2017).

**REGIONAL VARIATIONS IN VIRULENCE (PRV1)**

In Norway, most farmed Atlantic Salmon become PRV positive, but only some develop disease. This does not appear to be dependent on systemic PRV load, and it is not clear why some farms experience high losses due to HSMI while others do not. Nevertheless, clinical outbreaks of HSMI in farmed Atlantic Salmon of Norway are reasonably common (Kongtorp et al., 2004a; Kongtorp et al., 2004b; Kongtorp et al., 2006; Palacios et al., 2010), and laboratory challenge trials have demonstrated a clear ability for PRV to cause severe heart lesions (Wessel et al., 2017). Indeed, laboratory challenge trials in Norway routinely generate severe heart lesions in accompaniment with occasional skeletal muscle lesions similar to those observed on HSMI diseased salmon farms (Kongtorp et al., 2004b; Kongtorp and Taksdal, 2009; Mikalsen et al., 2012; Finstad et al., 2014; Lund et al., 2017).



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In Pacific Canada, there is a strikingly divergent relationship regarding PRV and its association with disease. PRV appears to be highly prevalent in farmed Atlantic Salmon of Pacific Canada (Marty et al., 2015); yet, a clinical outbreak of HSML as described in Norway (Kongtorp et al., 2004a; Kongtorp et al., 2004b) has never been reported. Two subclinical farm-level cases of HSML-like disease have been suggested to date (Di Cicco et al., 2017; Polinski et al., *in press*), but unlike in Norway, this disease could not be transmitted to naïve fish in a laboratory setting (Polinski et al., *in press*). Indeed, PRV has failed to cause severe heart lesions or any severity of skeletal muscle inflammation following experimental challenge of Atlantic or Pacific salmon in Pacific Canada (Garver et al., 2016a; Polinski et al., *in press*; Zhang et al., *in press*). Ongoing laboratory investigations directly comparing PRV isolated in both Norway and the Eastern Pacific have also preliminarily identified that the PRV from the Eastern Pacific is of lower virulence to Norwegian Atlantic Salmon (Wessel et al., 2018a).

Host, virus, and environmental factors may all be responsible or contributing factors for this regional altered virulence of PRV. The relative contribution by each of these putative factors is currently unknown; however, there are at least three potentially significant phenotypic dissimilarities between Canadian and Norwegian PRV1 that have been revealed through laboratory challenge trials (Figure 2). First, despite the similarity of Pacific Canada PRV and Norwegian PRV to produce high load viremia, Pacific Canada PRV remains absent from the plasma (Polinski et al., *in press*) while Norwegian PRV can be detected at high loads in the plasma for up to six weeks following infection (Finstad et al., 2014; Wessel et al., 2017). Second, there is a considerable difference in scale regarding host recognition of PRV. Although direct comparisons between Canadian and Norwegian studies are limited, mean systemic and heart-specific antiviral responses increased no more than fivefold in Pacific Canada studies (Garver et al., 2016a; Polinski et al., *in press*; Zhang et al., *in press*) whereas in Norwegian challenges these genes increased 10-50 fold in the blood (Haatveit et al., 2017; Wessel et al., 2017) and more than 100 fold in the heart (Mikalsen et al., 2012). The comparative lack of antiviral response to Pacific Canada PRV compared to Norwegian PRV is further supported by the relative protection PRV has afforded fish challenged with a secondary virus (IHNV) in Norway (Vendramin et al., 2018) but not in Pacific Canada (Polinski et al., 2016). Lastly, in addition to the discrepancies concerning the severity of heart inflammation outlined above, the timing of PRV associated heart inflammation is also different between challenges conducted with PRV from these two countries. Specifically, by either injection or cohabitation exposure of PRV, heart inflammation (prevalence and severity) in Norwegian studies consistently begins around the time of peak systemic PRV load, reaches high severity 1-2 weeks later, and thereafter diminishes (Lund et al., 2017; Wessel et al., 2017). In contrast, increased prevalence of heart inflammation in Pacific Canada challenge trials did not occur until approximately 4 weeks after peak PRV systemic loads were reached and maintained high prevalence (although not severity) for prolonged periods of greater than 6-7 weeks (Polinski et al., *in press*; Zhang et al., *in press*). All challenges were conducted at approximately the same temperature ((10-12°C).

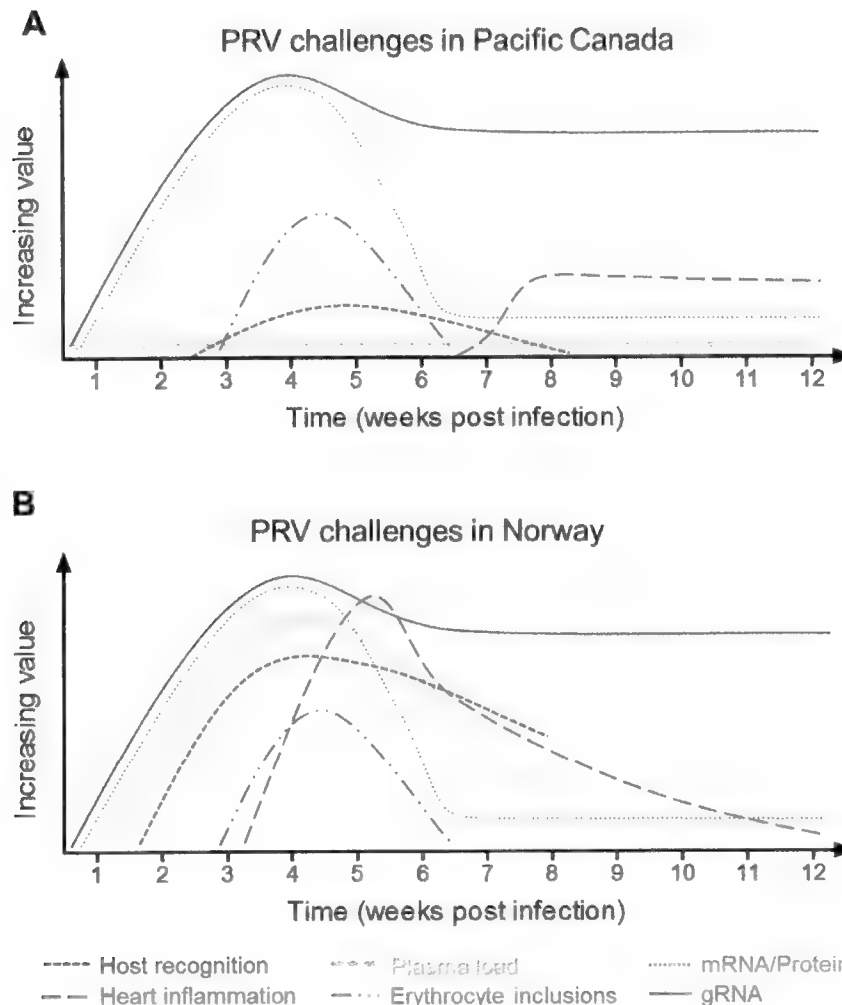


Figure 2. Contrast summary for trends in PRV phenotypic infection dynamics between Norway and Canada laboratory challenge of Atlantic Salmon (taken from Polinski et al., in press). In comparing challenge trials conducted in (A) Pacific Canada (Garver et al., 2016a; Polinski et al., in press; Zhang et al., in press) with results from similar challenge trials conducted in (B) Norway (Mikalsen et al., 2012; Finstad et al., 2014; Haatveit et al., 2017; Wessel et al., 2017).

## TRANSMISSION DYNAMICS (PRV1)

### Routes of Entry

PRV has been demonstrated to spread horizontally (from fish to fish) during laboratory cohabitation studies where PRV infections become evident in 100% of naïve fish (Garver et al., 2016a; Wessel et al., 2017). The route by which PRV enters naïve hosts remains unclear; however, fecal-oral transmission is a hallmark of many reoviruses and the presence of PRV1 in feces of infected fish (Hauge et al., 2016) coupled with the demonstrated ability of PRV to infect naïve fish via anal intubation (Hauge et al., 2016) suggests fecal-oral transmission is at least one likely route for natural PRV entry. Experimental studies have also generated PRV infections



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following waterborne immersion (Kvamme et al., 2018). Given that direct horizontal transmission of PRV can readily be accomplished, vector-mediated transmission (e.g., via a multicellular parasite) would present an unnecessary step in spreading PRV. Currently there is no evidence to suggest a vector is needed for PRV transmission.

Although the primary mode of PRV transmission is almost certainly horizontal, it is probable, given the systemic nature of PRV infections, that PRV contamination of sexual fluids permits the potential for egg-associated vertical (from parent to egg) transmission. Freshwater hatcheries in both North America and Europe have become infected presumably via this method; however, a study following a population of Norwegian Atlantic Salmon brood fish and progeny from 2008 to 2011 found that PRV was not isolated from eggs collected from infected brood fish, suggesting that vertical transmission, if occurring, is likely playing a minor role in PRV spread, particularly in natural environments (Wiik-Nielsen et al., 2012).

### Shedding

PRV infected salmon are considered a main transmission source of virus; yet it remains unknown as to how long and at what rates PRV is shed from an infected fish. Cohabitation studies where naïve salmon were introduced at different stages of PRV infection revealed that Atlantic Salmon recently infected with PRV were capable of transmitting virus but those in persistent stages of infection had reduced or ineffectual transmission to the naïve cohabitants (Garver et al., 2016a; Polinski et al., *in press*). Therefore, it is inaccurate to presume that all PRV infected Atlantic Salmon are equally contagious and are likely to transmit virus. Laboratory studies in Pacific Canada have demonstrated that Atlantic Salmon were highly infectious after 4-6 weeks of becoming infected with PRV (Garver et al., 2016a) but horizontal transmission was reduced by 15 weeks (Polinski et al., *in press*) and could not be accomplished after 44 weeks despite the ongoing persistence of PRV (Garver et al., 2016a). Based on these studies, it is hypothesized that natural horizontal transmission primarily occurs between 3-15 weeks following infection, after which the potential for natural shedding becomes severely reduced (Polinski et al., *in press*).

### Environmental stability

It can be presumed that PRV maintains at least a minimum capacity to survive in water, as successful waterborne transmission has been demonstrated experimentally. Yet, the extent to which PRV can remain infectious in the natural marine environment remains unknown. Many environmental factors such as sunlight, organic load, and indigenous microbial populations can adversely affect virus stability to varying degrees dependent upon virus type (Pinon and Viallette, 2018). For instance, viruses surrounded with an envelope are generally more easily rendered inactive than viruses without an envelope (Fitzgibbon and Sagripanti, 2008). Being free of an envelope, PRV could be expected to have greater stability than, for example, the envelope containing aquatic virus infectious hematopoietic necrosis virus (IHNV) which showed markedly reduced infectivity within hours of being held in natural seawater (Garver et al., 2013). However, due to the fact that decay rates are highly conditional upon virus and environmental factors, survival studies specific to PRV are required to accurately define its duration of infectivity in seawater. To date, such studies have not been undertaken due to the lack of conventional culture methodologies to conveniently monitor and evaluate the infectivity of PRV. Furthermore, suitable proxy data is unavailable as viral stability measurements from culturable surrogates such as Chum Salmon aquareovirus have not been performed.

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### Infectious potential

Preliminary evidence using PRV1 from Pacific Canada suggests that  $\leq 10^3$  PRV particles are sufficient to initiate infection by intra-peritoneal injection in Atlantic Salmon (M. Polinski, unpublished data). The minimum dose required to establish infection by immersion or ingestion is unknown, but the route of virus exposure, the host condition, stock, and species involved are all likely to play a role in the infectious potential of PRV. For example, Sockeye Salmon have exhibited detectable levels of PRV in blood as early as five days post intra-peritoneal injection (Polinski et al., 2016) while Sockeye continually cohabitated with PRV infected Atlantic Salmon did not acquire PRV blood infections until the 4<sup>th</sup> week of cohabitation (Garver et al., 2016a). Further, sentinel Sockeye Salmon showed substantially lower prevalence and intensity of PRV infections than in sentinel Atlantic Salmon of an equivalent exposure group after 4 weeks of cohabitation (Garver et al., 2016a), indicating that Sockeye Salmon are less susceptible to PRV than Atlantic Salmon and may require a more lengthy exposure period or dose to become infected. For newly smolted Pink Salmon (*Onchorhynchus gorbuscha*) (1 g), waterborne exposure to either 100 or 1,000 purified PRV particles per mL for one hour was insufficient to initiate infection (n=20) while an equivalent dose of 1,000 purified particles administered via intra-peritoneal injection established PRV infection in 90% of fish (n=10), suggesting a low susceptibility of Pink Salmon to waterborne infection (Richard, Polinski, and Garver, unpublished data). Refractivity to PRV1 by immersion has also been preliminarily demonstrated in Sea Trout (*Salmo trutta*) relative to Atlantic Salmon in Norway (Kvamme et al., 2018). It is currently unknown if these reduced susceptibilities are dose and/or duration dependent.

### Farmed-to-wild salmon transmission

Given the linkage with PRV to HSMI in Norwegian Atlantic Salmon farming, investigations have been conducted in Norway to evaluate the transmission of HSMI and PRV between neighboring farms and to wild fish populations. Sequence comparisons of PRV variants collected from farm and wild salmon in Norway revealed that PRV genotypes are similar regardless of host origin, suggesting that virus exchange is occurring between wild and farmed populations in Norway (Garseth et al., 2013; Madhun et al., 2018). However, neither the directionality nor the mechanism(s) responsible for exchanging PRV between farmed and wild populations are currently known. It has been postulated that interactions between wild and escaped farmed salmon, specifically when wild salmon migrate through aquaculture areas, may serve as potential mechanisms of virus perpetuation (Garseth et al., 2013). Nevertheless, comparisons of PRV prevalence in wild adult salmon from regions of northern Norway with differing farming intensity and disease frequency showed no association between salmon farming and the prevalence of PRV infection in wild salmon (Madhun et al., 2018).

In western North America, the high genome homology between PRV1 isolates of farmed and wild salmon (Siah et al., 2015) suggests the presence of a common reservoir and/or exchange of virus between wild and farmed populations. Yet the contribution of salmon farms to potentially exchange PRV with wild fish is unclear. One study has hypothesized salmon farms may influence PRV prevalence in wild Pacific salmon after identifying a higher prevalence of PRV in wild salmon with a "high" exposure probability to salmon farms than in fish sampled from "low" farm-exposure regions (Morton et al., 2017); although it must be noted that the categorization for low and high farm exposure used in this study is highly speculative. In contrast, a study that compared prevalence of PRV in Coho Salmon from Alaska (an area devoid of open net pen salmon aquaculture) to Coho Salmon from British Columbia (where salmon farms are present) identified no significant difference in PRV prevalence, suggesting salmon farming was contributing negligibly to PRV prevalence in these wild Coho stocks (Marty et al., 2015).

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Chinook Salmon also screened for PRV in Alaska (Purcell et al., 2018) similarly showed analogous prevalence and stock variability for PRV detection to Chinook Salmon of British Columbia (Marty et al., 2015) (Table 2); also suggesting that salmon farms are having minimal direct impacts to PRV prevalence in Chinook of the Eastern Pacific. Undoubtedly a multitude of factors are responsible for influencing PRV prevalence in wild salmon, which is clearly evident by the fact that PRV prevalence varies considerably across host species and even between cohorts of a particular species (Purcell et al., 2018). Consequently, longer time scale monitoring efforts in conjunction with molecular epidemiology studies are needed to fully appreciate the drivers of PRV infection in salmon population of western North America.

## PREVALENCE IN WESTERN NORTH AMERICA

Molecular diagnostic screening has been applied in numerous surveillance programs that have identified the presence of PRV among farmed and wild salmon collected over the geographic range spanning Alaska to Washington. Analyses of archived salmon samples from 1974-2008 from British Columbia also revealed a long-term and common presence of PRV1 in the Eastern Pacific with positive detections identified in samples dating back to 1987 and possibly as early as 1977 (Marty et al., 2015). Both farmed and wild fish stocks have been shown to become infected.

### Farmed Atlantic Salmon

Once PRV becomes present on a salmon farm, it is expected to reach 100% prevalence within the population (Di Cicco et al., 2017; Polinski et al., *in press*). In a temporal study of PRV at one Atlantic Salmon farm site in British Columbia, PRV was first detected 3 to 4 months following seawater entry and peaked at 100% several months later (Di Cicco et al., 2017). A second study also identified 100% PRV prevalence at a different British Columbia Atlantic Salmon farm site after fish had spent 3 months at sea (Polinski et al., *in press*). More recently, a sampling survey of dead or dying fish collected in all aquaculture zones of BC demonstrated that time-at-sea was a significant predictor for the detection of PRV in Atlantic Salmon with prevalence increasing up to 18 month post seawater entry and declining thereafter (Laurin et al., 2019). Additionally, current ongoing research examining PRV prevalence on 13 Atlantic Salmon farms spread across British Columbia found that fish at all 13 sites became infected with PRV with a general onset within 100 and 200 day post seawater entry that was independent of location or time of stocking. Further, following initial infection, all 13 farms reached 100% infection prevalence within 100 days of first detection (Polinski and Garver, unpublished data).

### Wild Pacific salmon

Either through experimental or natural infection, all five species of North American Pacific salmon have been shown to be capable of supporting PRV infections; however, surveys of wild Pacific salmon demonstrate that PRV prevalence can vary dramatically between species and stock. Across multiple independent surveys of Pacific salmon and trout, PRV was consistently detected at higher prevalence in Chinook and Coho salmon as compared to Chum (*O. keta*), Pink, Sockeye, and steelhead Trout (*O. mykiss*). The overall prevalence for Chinook and Coho salmon identified within these studies reached approximately 6% and 9%, respectively, while PRV prevalence in Pink Salmon remained below 4%, Sockeye around 1.4%, and Chum as well as steelhead less than 1% (Table 2).

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Table 2. PRV1 prevalence in North American Pacific salmon and trout species sampled in Alaska, British Columbia, and Washington. Numbers in parentheses represent the PRV positive fish per total number of fish sampled.

Species	PRV1 surveillance studies						Overall prevalence
	(Marty et al., 2015)	(Purcell et al., 2018)	(Morton et al., 2017)	S. Johnson unpublished	Studies using Fluidigm BioMark™ assay <sup>a</sup>	Unpublished student theses <sup>b</sup>	
Sockeye Salmon	0.3% (1/371)	0.0% (0/394)	9.3% (21/225)	0.0% (0/717)	1.6% (67/4215)	1.0% (8/771)	1.4% (97/6693)
Chinook Salmon	8.8% (6/68)	4.0% (19/480)	34.3% (34/99)	4.4% (54/1232)	2.8% (9/325)	2.4% (1/41)	5.5% (123/2245)
Coho Salmon	7.6% (9/118)	11.8% (56/473)	26.1% (18/69)	4.5% (16/356)	1.7% (1/61)	--	9.3% (100/1077)
Pink Salmon	0.0% (0/313)	0.4% (1/243)	25.0% (27/108)	0.0% (0/70)	--	--	3.8% (28/734)
Chum Salmon	0.0% (0/101)	0.0% (0/287)	7.5% (5/67)	0.0% (0/135)	--	--	0.8% (5/590)
Steelhead Trout	--	0.3% (1/375)	28.6% (4/14)	--	--	1.0% (3/303)	0.9% (5/553)
Cutthroat Trout	--	--	50.0% (8/16)	--	--	trout combined	--
Dolly Varden Trout	--	--	10.0% (1/10)	--	--	--	--

<sup>a</sup>(Jeffries et al., 2014; Miller et al., 2014; Bass et al., 2017; Teffer et al., 2017; Nekouei et al., 2018; Teffer et al., 2018; Thakur et al., *in press*)

<sup>b</sup>(Furey, 2016; Healy, 2017; Hrushowy, 2018; Stevenson, 2018)

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Specific to Sockeye Salmon, 12 independent studies cumulatively indicate that the majority of samples positive for PRV nucleic acid were collected from returning adults (Table 3). A cumulative 0.3% (12/3911) of fry and juvenile fish were positive for PRV whereas 2.9% (85/2912) of returning adults were positive. This data also suggests that most PRV infections occurred at sea. PRV was detected on or in out-migrating smolts collected at the mouth of Queen Charlotte Strait and within the southern Queen Charlotte Sound (after presumed northward migration through the Discovery Islands/Johnson Strait) at a prevalence of 0.8% (7/833), whereas fry and parr had a nominally lower prevalence of 0.4% (4/1072) in freshwater, with most detections (3) occurring in a population of fry from Oweekeno Lake that is not associated with the Fraser River (Table 3). Similarly, the majority of PRV detections in adult Sockeye Salmon (63/85 total positives) occurred in one study which screened gill biopsies of returning adult fish migrating southwards through the Johnston Strait/Discovery Islands (Miller et al., 2014). Interestingly, liver samples taken at the same time of gill biopsies as well as subsequently in the Fraser River were negative for PRV; suggesting that the PRV on or in the gill tissues of these fish did not represent systemic infections nor did systemic infections likely develop before returning fish reached their spawning grounds.

Within the Fraser River, PRV has been detected in at least five stocks of Sockeye Salmon (Table 4), although sampling for many stocks has been limited and the single Nadina River sample positive for PRV nucleic acid was considered questionable by the authors (Marty et al., 2015). Further, it should again be noted that 63/68 positive PRV detections occurred as a result of gill biopsies taken from returning adults passing through the Johnstone Strait/Discovery Islands which did not appear to develop systemic infections (Miller et al., 2014).

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Table 3. Detection of PRV in Sockeye Salmon of Alaska, British Columbia, and Washington by life stage. Numbers in parentheses represent the PRV positive fish per total number of fish sampled. Cumulative detection specific to Fraser River Sockeye Salmon (FRSS) stocks (where identified) is also presented; the 29 adult fish sampled in saltwater by Morton et al. are of unknown (possibly Fraser River) origin but not incorporated into the FRSS summary.

Data Source	Sockeye Salmon PRV prevalence				
	Fry	Parr/smolt		Adults	
	Freshwater	Freshwater	Saltwater	Saltwater	Freshwater
Marty et al. (2015)	--	0/30	--	--	1/341
Purcell et al. (2018)	--	--	--	--	0/394
Johnson (unpublished)	--	0/344	0/373	--	--
Morton et al. (2017)	--	1/1	3/90	0/29	17/105
Miller et al. (2014)	--	--	1/165	64 <sup>1</sup> /531	1/498
Teffer et al. (2017)	--	--	--	--	0/112
Thakur et al. ( <i>in press</i> )	--	--	--	--	0/652
Nekouei et al. (2018)	--	0/896	1/1110	--	--
Jeffries et al. (2014)	--	0/228	--	--	0/23
Stevenson (2018)	--	0/300	--	--	--
Furey (2016)	--	0/80	--	--	--
Hrushowy (2018)	3/89	--	3/205	--	2/97
<b>Totals</b>	<b>3.4% (3/89)</b>	<b>0.1% (1/1879)</b>	<b>0.4% (8/1943)</b>	<b>11.4% (64/560)</b>	<b>0.9% (21/2222)</b>
<b>Totals (FRSS only)</b>	<b>--</b>	<b>0.1% (1/1505)</b>	<b>0.2% (2/1258)</b>	<b>12.1% (64/531)</b>	<b>1.3% (19/1431)</b>

<sup>1</sup>63/155 detections of PRV from gill biopsies but 0/57 detections in liver tissues collected at same location

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541 *Table 4. Distribution of PRV detection across Fraser River Sockeye Salmon stocks (Jeffries et al., 2014;*  
542 *Miller et al., 2014; Marty et al., 2015; Furey, 2016; Morton et al., 2017; Teffer et al., 2017; Nekouei et al.,*  
543 *2018; Stevenson, 2018).*

Stock screened for PRV	PRV screening results	
	Juveniles	Adults
Bowron	0/9	--
Cultus	1/62	--
Weaver	0/8	--
Portage	0/35	--
Early Stuart, Late Stuart & Misc. <sup>1</sup>	0/4	1/191
Quesnel	0/22	0/297
Horsefly	0/148	--
Mitchell	0/119	--
Blue Lead	0/1	--
Wasko-Roaring	0/16	--
Nahatlatch River	0/16	--
Fennell	0/1	--
Thompson	0/75	--
Raft	0/18	--
Upper Barrier	0/3	--
Birkenhead	0/77	0/11
Scotch	0/72	0/8
Seymour	0/134	--
Adams	1/370	0/2
Shuswap	0/398	49/304
Eagle	0/6	--
Little	0/5	--
Nadina	0/60	1 <sup>2</sup> /60
Dolly Varden	0/86	--
Chilliwack Lake	0/34	--
Stellako	0/137	0/10
Gates	0/65	0/19
Big Silver	0/4	--
Pitt	0/79	--
Harrison	--	0/103
Chilko	0/1018	15/250

544 <sup>1</sup> This includes juvenile fish sampled from Sandpoint Creek, Five Mile Creek, Middle River, and Dust-  
545 Sinta Creek (n=1 per stock).

546 <sup>2</sup> Positive detection of PRV nucleic acid in only one of two technical replicates which was noted as  
547 inconclusive by the authors (Marty et al., 2015).

## CARDIOPATHY OF SALMON

### CAUSATIVE FACTORS

Cardiopathy refers to diseases of the heart that affects contractive functions and decreased capacity to circulate blood. These diseases have many causes and, in association with the global production of farmed salmon, a variety of cardiopathies have been described. Some occur as a result of non-transmissible conditions such as during cardiac remodeling and expansion due to chronic hypoxia stress (Simonot and Farrell, 2007) or as a result of congenital mutation (Becker et al., 2011). However, cardiopathy can also occur due to infectious and transmissible microbes. Specific to salmon, at least eight infectious agents are known to cause cardiopathic disease, although high-load systemic infections of virtually any moderately virulent pathogen has the potential to inflict damage to heart tissues:

- *Renibacterium salmoninarum* (Bruno, 1986)
- *Piscirickettsia salmonis* (Olsen et al., 1997)
- *Kudoa thyrsites* (Moran et al., 1999)
- *Ichthyophonus hoferi* (Kocan et al., 2006)
- *Yersinia ruckeri* (Rucker, 1966)
- Salmonid alpha virus (SAV) (Wiik-Nielsen et al., 2016)
- Piscine myocarditis virus (PCMV) (Haugland et al., 2011)
- Piscine orthoreovirus (PRV) (Wessel et al., 2017)

Of these, six are endemic to British Columbia: *I. hoferi*, *K. thyrsites*, *P. salmonis*, *R. salmoninarum*, *Y. ruckeri* and PRV. In the event that the causative agent of a heart disease is not clearly identifiable, a diagnosis of idiopathic cardiopathy is assigned.

### PREVALENCE AND IMPACT IN BRITISH COLUMBIA

#### General cardiopathy

Mild cardiopathy is prevalent in farmed salmon of British Columbia; however, severe cardiopathy impairing heart function is rare. Between 2006 and 2018, the Fish Health Auditing and Surveillance Program (FHASP) conducted by DFO Aquaculture Management Division has evaluated all major organs of nearly 6,000 Atlantic and 800 Pacific (majority Chinook; some Coho) salmon net-pen farming mortalities by histopathology including heart tissues. Mild to moderate cardiopathy occurred in 61% of Atlantic and 41% of Pacific salmon mortalities sampled during this period. However, this cardiopathy, mainly epi- and endocarditis, does not compromise heart or respiratory function (Lund et al., 2017; Zhang et al., *in press*) and is not expected to adversely affect salmon health. Moderate to severe cardiopathy with a putative ability to negatively affect heart functioning was diagnosed in 7% and 3% of Atlantic and Pacific salmon mortalities, respectively. The severity was sufficient to be suggested as a putative cause or contributing factor to death in less than 3% of both Atlantic and Pacific salmon species (Figure 3). These percentages are representative of sites specific to the Discovery Islands region and are consistent with other independent studies which have corroborated the relatively widespread occurrence of generally mild cardiopathy in British Columbia salmon with little evidence for its contribution to morbidity or mortality over the past decade (Marty et al., 2015; Di



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Cicco et al., 2017). This is also consistent with previous diagnoses of prevalence for severe cardiopathy in farmed salmon from the early 1990's (Brackett et al., 1990; Brackett et al., 1991; Brackett and Newbound, 1992; Brackett et al., 1992); suggesting that cardiopathy has likely caused or contributed to less than 0.4% cumulative mortality in farmed salmon in BC over the past 25 years. The proportion of this cardiopathy that is attributable to infectious diseases and specifically PRV is unknown, although multiple transmissible and non-transmissible factors are indicated to be involved in addition to PRV (Figure 3).

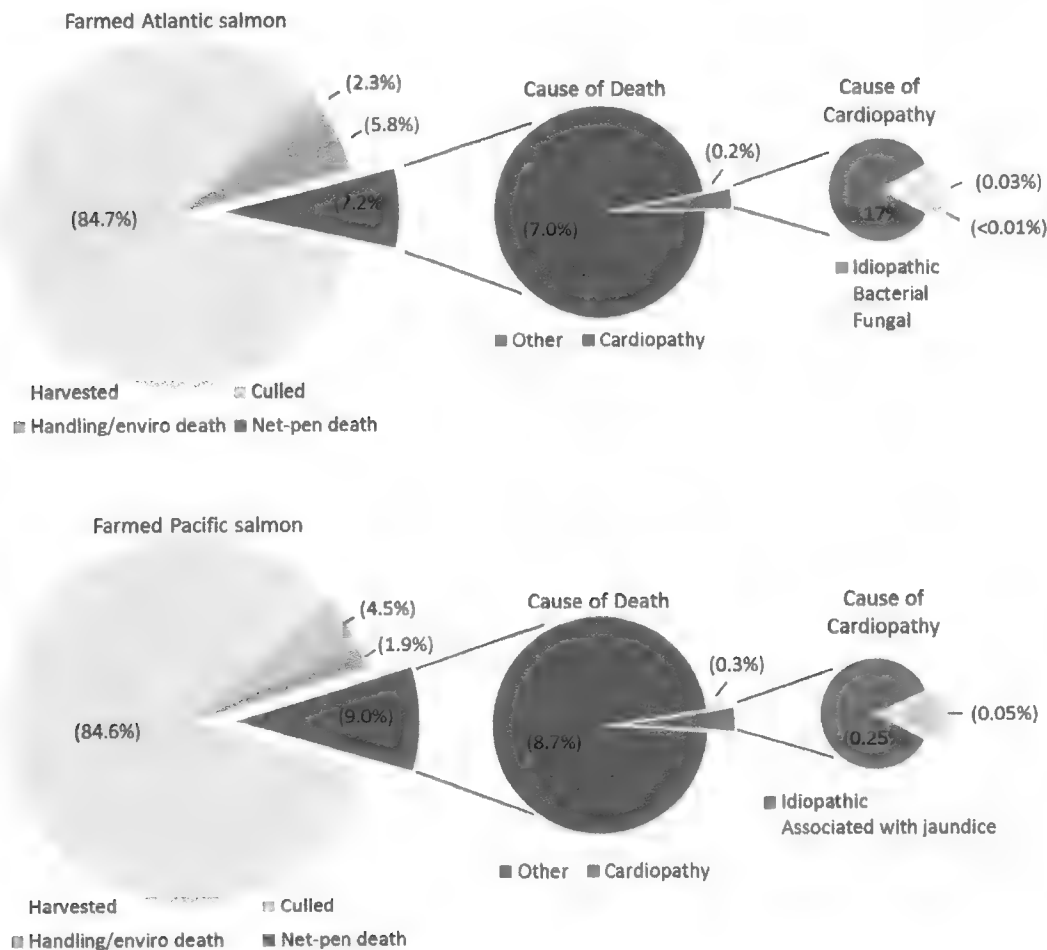


Figure 3. Cardiopathy as a marker of death in farmed salmon of British Columbia. Cumulative percent mortality of stocked fish per annum presented in left pie charts are extrapolated from the mean monthly mortalities reported across salmon farming industries between 2012-2018. Putative cause of death diagnoses from net-pen mortalities not associated with culling, handling, or environmental causes (e.g., low dissolved oxygen) is presented in the center pie charts based on FHASP data collected between 2006 and 2018. The putative causes of cardiopathy in these instances are presented in the right pie charts. All percentages are relative to total number of net-pen Atlantic or Pacific salmon stocked per annum during this time period.

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The prevalence of cardiopathy in wild Pacific salmon is relatively unknown; however, a survey of 204 wild salmon of British Columbia in 2013 that included Pink, Chum, Chinook, Coho, and Sockeye salmon diagnosed mild cardiopathy in 12 fish (5.9%) but failed to identify significant (severe) cardiopathic disease (Marty et al., 2015). Similarly, severe cardiopathy that occurs in farmed Atlantic Salmon in Norway (e.g., HSMI, CMS and PD) has not been detected in wild Atlantic Salmon (Garseth et al., 2013), suggesting environmental components specific to intensive culture likely enhance the prevalence and severity of cardiopathy in salmon.

### HSMI

The term HSMI, although foundationally descriptive, has evolved considerably in meaning over the past decade. Before a causative agent was known, the original diagnosis of HSMI was founded on a set of distinct clinical disease characteristics in Norwegian Atlantic Salmon farms during episodes of morbidity and/or mortality for which histopathology was used to confirm and differentiate this condition from other similar diseases; e.g., pancreatic disease or cardiomyopathy syndrome. By this original case definition, HSMI has never been reported in British Columbia:

*"Affected fish are anorexic and display abnormal swimming behaviour. Autopsy findings typically include a pale heart, yellow liver, ascites, swollen spleen and petechiae in the perivisceral fat. Diagnosis of HSMI is presently based on histological examination. HSMI is characterised by extensive panmyocarditis and myositis, particularly involving red skeletal muscle. Morbidity may be very high, while mortalities are variable and may reach 20% in affected cages."* (Kongtorp et al., 2004a).

Following the discovery of PRV in association with HSMI in Norway in 2010 (Palacios et al., 2010) and the subsequent demonstrated ability for PRV to cause severe heart inflammation (Wessel et al., 2017), the diagnosis for HSMI, although still exclusively based on histopathology, is generally accepted to be initiated by PRV. Many subclinical infections of HSMI have now been diagnosed in Norway, some even without the evidence of skeletal muscle inflammation, and although environmental and/or host contributing factors may explain the often exacerbated severity of HSMI in a field relative to laboratory setting, PRV appears to be the sole infectious agent associated with the unique set of histopathological criteria that defines HSMI in Norway (Palacios et al., 2010; Wiik-Nielsen et al., 2016; Wessel et al., 2017). To our knowledge, HSMI has never been used to classify a disease state in Norway where PRV has been confirmed to be absent.

Two recent studies from Pacific Canada have also used the term HSMI to classify subclinical heart disease of farmed Atlantic Salmon based on histopathology in accordance with their own definitions similar to those previously reported in Norway – namely, moderate to severe heart inflammation sometimes accompanied by skeletal muscle inflammation (Di Cicco et al., 2017; Di Cicco et al., 2018). Although the presumed commonality for the heart and skeletal muscle lesions in these Canadian studies relative to HSMI diagnosed in Norway is the causation by PRV, there is far less evidence in Canada to support that PRV is indeed the key component for initiating this relatively rare disease state; particularly given that these modified definitions have occasionally been observed in the absence of PRV (Marty et al., 2015; Di Cicco et al., 2018). Consequently, if HSMI diagnosis is based solely on histopathological heart lesions which can occur in the absence of PRV, then PRV cannot be assumed to be *the* causative agent of the disease, but rather one of multiple stand-alone or synergistic putative factors. Thus, if using the definition proposed by Di Cicco et al. (2017), HSMI in British Columbia can likely be used interchangeably with the terms 'moderate to severe cardiopathy' or 'idiopathic cardiopathy' as

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described above. Throughout this review, we use the term HSMI only in cases which fit those described by Wiik-Nielsen et al (2016) where PRV appears to be the most likely primary causative factor.

**ANEMIA OF SALMON**

**CAUSATIVE FACTORS**

Anemia is a condition marked by a deficiency in red blood cells and/or haemoglobin that results in a reduced ability for blood to transport oxygen. Many factors can cause or contribute to anemia in fish including nutrient deficiencies, toxic agents and infectious pathogens (Witeska, 2015). Specific to salmon, at least eight pathogenic organisms (including viruses, bacteria, and external parasites) are known to directly or indirectly contribute to anemia although this is almost certainly not a comprehensive list:

- Infectious salmon anemia virus (ISAV) (McBeath et al., 2015)
- Infectious hematopoietic necrosis virus (IHNV) (Amend and Smith, 1975)
- Piscine orthoreovirus (PRV) (Takano et al., 2016)
- *Aeromonas* sp. (Řehulka, 2002)
- *Flavobacterium columnare* (Řehulka and Minařík, 2007)
- *Vibrio anguillarum* (Harbell et al., 1979)
- *Ichthyophthirius multifiliis* (Abdel-Hafez et al., 2014)
- *Tetracapsuloides bryosalmonae* (Hoffmann and Lommel, 1984)

Of these, there are five relevant to salmon of British Columbia that reside in saltwater: IHNV, PRV, *Aeromonas* sp, *F. columnare* and *V. anguillarum*. However, although PRV is listed here, it must be noted that the only PRV isolate to induce anemia in salmon through experimental infection to date is that of PRV2 from Japan (Takano et al., 2016).

It also must be acknowledged that the measures used to assess anemia (erythrocyte count, haemoglobin concentration and hematocrit) are at best indirect measures because they do not necessarily imply a lack of sufficient oxygen delivery. This is highly important when considering that, unlike for mammals, these measures can fluctuate substantially in healthy populations of fish. In salmon, the packed erythrocyte volume (hematocrit) can vary as much as 40% between individuals within a single cohort population (i.e., hematocrit ranging from 40-65% of total blood volume) without significant correlation to respiratory functioning (Zhang et al., *in press*). For salmon, it has been suggested that functional anemia occurs when hematocrit drops below approximately 20-25% total blood volume (Simonot and Farrell, 2007; Clauss et al., 2008), although this estimate will likely vary depending on a variety of environmental and host-specific factors. Thus, clinical symptoms such as lethargy or other signs of morbidity and/or mortality are important characteristics for identifying fish which are truly anemic (i.e., have a loss in respiratory function) relative to those which may have a reduced hematocrit or haemoglobin relative to what is 'typical' for the species but remain physiologically uncompromised.

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**IMPACT AND PREVALENCE IN BRITISH COLUMBIA**

Unexplained anemia (i.e., with the potential to be caused by an unknown pathogen such as PRV) is rarely documented in salmon of British Columbia. There were no veterinary diagnoses indicative of anemia in farmed Atlantic Salmon made as part of the FHASP within DFO between 2011 and 2017 that included 663 site visits and 4,344 sampled specimen carcasses. There were also no diagnoses of anemia in farmed Coho Salmon during this period (17 site audits involving 75 collected specimens). In farmed Chinook Salmon, a condition referred to as Jaundice Syndrome was diagnosed by the FHASP veterinarian for 7 out of 479 carcasses (1.5%) in 5 of 95 site audits during this seven year period. Jaundice Syndrome is characterized by yellow discoloration of the skin resulting from excessive bilirubin in the blood due to red blood cell breakdown. Substantial red blood cell breakdown is needed to cause jaundice and can therefore be used as a proxy for identifying current or recent anemia. The prevalence of jaundice/anemia reported during the FHASP is similar to that reported previously in farmed Chinook Salmon of BC (< 1.5% jaundice-associated mortality per production cycle) that appears to have a seasonal (cold water temperature) component (Garver et al., 2016b). This seasonality is at least partially substantiated by a focused study involving 210 FHASP samples collected from Chinook Salmon in 2011-2013 by Di Cicco et al. (2018), where the authors noted what they classified as jaundice syndrome based on their own definitions using histopathology (a more liberal classification than previously used by government or industry veterinarians) in 14 fish (6.7%) that was restricted to two specific sampling events. The occurrence of unexplained anemia in wild Chinook Salmon or any other Pacific salmon species in British Columbia is unknown.

**RELATIONSHIP OF PRV AND DISEASE IN BRITISH COLUMBIA**

**ATLANTIC SALMON**

Experimental challenge trials using PRV1 in Pacific Canada Atlantic Salmon have resulted in extreme PRV blood infections, but either failed to generate notable pathology (Garver et al., 2016a) or induced only minor to moderate heart inflammation – specifically, epi- and endocarditis (Polinski et al., *in press*; Zhang et al., *in press*). Further, the increased prevalence of minor heart inflammation induced by PRV (when it occurred) in these experiments was demonstrated to be inconsequential to normal heart and respiratory functioning (Zhang et al., *in press*). However, a correlation of PRV to moderate to severe heart inflammation has been proposed in a field environment based on a longitudinal study of one farm site with a high transient presence of moderate to severe cardiopathy (Di Cicco et al., 2017). The visualization of PRV in diseased hearts in this study in conjunction with activation of host cellular antiviral response pathways strongly suggested that PRV was contributing to the severity of cardiopathy observed. Thus, taken together, these studies suggest that PRV has the potential to exacerbate instances of severe cardiopathy in net-pen farmed Atlantic Salmon in British Columbia in some case and may be a contributing etiological factor in establishing at least some instances of this relatively rare disease.

**PACIFIC SALMON**

Two PRV experimental challenge studies have been published exploring the disease causing potential of PRV1 to Sockeye Salmon; both failed to identify an association with PRV and disease (Garver et al., 2016a; Polinski et al., 2016). A third study has also recently been completed with Sockeye Salmon that failed to generate anemia or notable pathology in heart,

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kidney, or liver tissues of infected fish; and, in the same manner as assessing physiological impacts of PRV on Atlantic Salmon (Zhang et al., *in press*), demonstrated these infections to be inconsequential to Sockeye physiological respiratory functioning (Polinski et al., manuscript in preparation). For adult Chilko or Shuswap Sockeye Salmon returning to the Fraser River, it was identified that the presence of PRV on or in the gills of fish migrating through Discovery or Juan De Fuca sea channels had no significant effect on the likelihood that they would reach their spawning grounds (Miller et al., 2014). Coho Salmon challenged with PRV harvested from infected farmed Atlantic Salmon in British Columbia also failed to acquire notable pathology or anemia despite attaining substantial PRV blood infections (Winton et al., manuscript in preparation). Lastly, experiments attempting to passage jaundice syndrome in Chinook Salmon in association with PRV failed to passage the disease despite successfully passing PRV (Garver et al., 2016b). Nevertheless, similar to field observation of PRV contributing to severe cardiopathy in Atlantic Salmon, PRV has been visualized in diseased tissues of farmed Chinook Salmon experiencing Jaundice Syndrome (Di Cicco et al., 2018) which would suggest that PRV is capable of contributing to jaundice/anemia and may be part of a more complex aetiology for establishing this rare disease state in Chinook Salmon.

## DISEASE PREVENTION

In British Columbia, there has been no data to suggest PRV adversely affects aquaculture production of salmon. In Norway, however, HSML is considered one of the most significant infectious diseases affecting Atlantic Salmon aquaculture (Hjeltnes B et al., 2017; Marine Harvest, 2017) and a number of strategies are being explored to mitigate this disease. Two experimental vaccination studies have been conducted; one using a formalin killed PRV vaccine (Wessel et al., 2018b) and one using a DNA vaccine expressing the non-structural proteins of PRV (Haatveit et al., 2018). Both demonstrated moderate protection against HSML, although neither were protective against PRV infection. In addition to vaccination, work towards establishing a "HSML-resistant" Atlantic Salmon strain has been undertaken (AquaGen, 2017), although similar to the vaccines mentioned above, these fish do not appear to be refractory to PRV but rather only HSML (Emilsen et al., 2017). Furthermore, use of specific feed formulations, similar to the other treatments, has shown promise at reducing the effects of HSML primarily through dietary immunomodulation but is not successful at eliminating PRV infections (Grammes et al., 2012; Martinez-Rubio et al., 2012).

## SUMMARY

There has been a great deal of knowledge gained regarding the virology and ecology of PRV following its discovery nine years ago; and much of that knowledge has direct or indirect relevance for assisting in the assessment of risk to Fraser River Sockeye Salmon posed by the occurrence of PRV on Atlantic Salmon farms. The most critical research findings that can aid this risk assessment are summarized here:

- PRV is ubiquitous and highly prevalent in net-pen farmed salmon of British Columbia; it is also widely distributed in wild Pacific salmon but with less prevalence and species/stock-specific variation.
- Farmed and wild salmon of British Columbia appear most likely to become infected with PRV as adults in saltwater although freshwater infections of fry/parr can and have occurred.

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- Infections with PRV in British Columbia generate high-load blood infections in both Atlantic and Pacific salmon species but have failed to generate notable (moderate to severe) disease following experimental infection in any species challenged (Sockeye, Chinook, Coho, Pink and Atlantic).
- PRV is of lower virulence to Atlantic Salmon in British Columbia relative to PRV infections of Atlantic Salmon in Norway; both host and virus specific factors are likely involved in this altered virulence.
- Infections of PRV in Atlantic and Sockeye salmon of British Columbia have been demonstrated as inconsequential to respiratory function in the absence of notable (moderate to severe) pathology.
- The severity of a systemic PRV infection is not indicative of whether a salmon will or will not develop a notable disease.
- PRV may contribute to or be a possible etiological component of severe heart inflammation in farmed Atlantic Salmon or jaundice/anemia syndrome in farmed Chinook Salmon in British Columbia; both conditions appear rare and likely have complex etiologies.
- There is currently no evidence to suggest that PRV causes disease in Sockeye Salmon, which appear less susceptible to infection relative to Atlantic Salmon in British Columbia.

Despite substantial gains in our understanding about PRV, there are also knowledge gaps concerning PRV virology and ecology that leave critical uncertainties. The environmental source(s) and transmission potential of PRV in ocean environments are unknown. Specifically, there is no current data on environmental shedding (quantity or duration) or the minimum exposure load (quantity or duration) to establish an infection in any salmon species. There is also a current lack of understanding for why PRV can show higher virulence in some instances compared to others. Lastly, in the instances where PRV has been linked to disease in farmed salmon, it is as yet unclear as to whether all host, environment, and viral specific factors of these diseases can manifest in the natural environment in British Columbia.

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**PRV and Associated Disease**

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## **Canadian Science Advisory Secretariat (CSAS)**

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### **Assessment of the risk to Fraser River Sockeye Salmon due to Piscine Orthoreovirus (PRV) on Atlantic Salmon farms in the Discovery Islands area, British Columbia**

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### **Foreword**

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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## GLOSSARY

**Acute:** characterized by a short and relatively severe course

**Cardiopathy:** diseases of the heart (including all vascular, epicardial, and myocardial conditions) that affect contractive functions and decrease the capacity of the heart to circulate blood

**Cardiomyopathy:** disturbance or disease of the heart muscle

**Chronic:** a disease condition that is persistent or long lasting

**Clinical:** outward appearance of a disease in a living organism

**Disease:** condition in which the normal function or structure of part of the body or a bodily function is impaired

**Epidemiological unit:** a group of animals that share approximately the same risk of exposure to a pathogenic agent with a defined location

**Fish Health Event (FHE):** a suspected or active disease occurrence within an aquaculture facility that required the involvement of a veterinarian and any measure that is intended to reduce or mitigate impact and risk that is associated with that occurrence or event

**Fomite:** an inanimate object capable of transmitting a disease (e.g., contaminated net or boat)

**Genogroup:** phylogenetically distinct group or cluster

**Horizontal transmission:** fish to fish transfer of a pathogen

**HSMI:** a heart and skeletal muscle inflammatory disease of farmed Atlantic Salmon characterized by cellular epicarditis, moderate-to-severe inflammation and necrosis (especially in the ventricle with inflammation predominant) where inflammation of the red skeletal muscle is a supportive finding; and PRV is evidenced to be a major etiological factor

**HSMI-like:** inflammatory heart disease as characterized for HSMI but with questionable etiology

**Incubation period:** time between host infection by a pathogenic organism and appearance of the first signs of disease

**Infection:** growth of pathogenic microorganisms in the body, whether or not body function is impaired

**Infection pressure:** concentration of infective pathogens in the environment of susceptible hosts

**Mortality event:** fish mortalities equivalent to 4000 kg or more, or losses reaching 2% of the current facility inventory, within a 24 hour period; or fish mortalities equivalent to 10,000 kg or more, or losses reaching 5%, within a five day period

**Outbreak:** the occurrence of one or more cases of a disease than would normally be expected in an epidemiological unit over a given period of time

**Prevalence:** number of hosts infected with a pathogen (*infection prevalence*) or affected by a disease (*disease prevalence*) expressed as a percentage of the total number of hosts examined for that pathogen (or disease) in a population at a specific time

**Subclinical:** insufficient signs to cause classical identifiable disease

**Sublethal:** insufficient to cause death

**Susceptible species:** a species in which infection has been demonstrated by the occurrence of natural cases or by experimental exposure to the pathogenic agent that mimics natural transmission pathways

**Vector:** living organism that has the potential to transmit a disease, directly or indirectly, from one animal or its excreta to another animal (e.g., personnel, wildlife, etc.).

PRV risk assessment

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## **ABSTRACT**

(To come)

**Évaluation du risque pour le saumon rouge du fleuve Fraser  
que représente le transfert du orthoréovirus pisciaire  
à partir des fermes de saumon atlantique situées dans  
la région des îles Discovery (Colombie-Britannique)**

## **RÉSUMÉ**

(To come)



## INTRODUCTION

Fisheries and Oceans Canada (DFO) has a regulatory role to ensure the protection of the environment while creating the conditions for the development of an economically, socially and environmentally sustainable aquaculture sector. Within this overall objective, DFO's goal for aquaculture is to ensure that fish and their habitats are protected using avoidance, mitigation, monitoring and compliance approaches that are aligned with the potential risk to the environment.

It is recognized that there are interactions between aquaculture operations and the environment (Grant and Jones, 2010; Foreman et al., 2015b). One interaction is the risk to wild salmon populations resulting from the potential spread of infectious diseases from Atlantic Salmon (*Salmo salar*) farms in British Columbia (BC) (Cohen, 2012a).

DFO Aquaculture Management Division requested formal science advice on the risk of pathogen transfer from Atlantic Salmon farms to wild fish populations in BC. Given the complexity of interactions between pathogens, hosts and the environment, DFO is delivering the science advice through a series of pathogen-specific risk assessments to be followed by a synthesis.

This document assesses the risk to Fraser River Sockeye Salmon attributable to piscine orthoreovirus (PRV) from Atlantic Salmon farms in the Discovery Islands area in BC. Risk posed to other wild fish populations and related to other fish farms, pathogens, and regions of BC will be determined through subsequent analyses and are consequently not included in this document.

## BACKGROUND

This risk assessment is conducted under the DFO Aquaculture Science Environmental Risk Assessment Initiative (hereinafter referred to as the Initiative) implemented as a structured approach to provide science-based risk advice to further support sustainable aquaculture in Canada. Furthermore, to ensure consistency across risk assessments conducted under the Initiative, the Aquaculture Science Environmental Risk Assessment Framework (hereinafter referred to as the Framework) outlines the process and components of each assessment.

The Framework ensures the delivery of systematic, structured, transparent and comprehensive risk assessments. It is consistent with international and national risk assessment frameworks (GESAMP, 2008; ISO, 2009) and has been validated through multiple peer-reviewed processes (Mimeault et al., 2017; Mimeault et al., *in review-a*; Mimeault et al., *in review-b*; Mimeault et al., *in review-c*; Mimeault et al., *in review-d*). The Framework includes the identification of management protection goals, a problem formulation, a risk assessment and the generation of science advice. The management protection goals and problem formulation were developed in collaboration with DFO's Ecosystems and Oceans Sciences and Ecosystem and Fisheries Management sectors and approved by Aquaculture Management Division.

The Framework also comprises risk communication and a scientific peer-review through DFO's Canadian Science Advisory Secretariat (CSAS) that includes scientific experts both internal and external to DFO. Further details about the Initiative and the Framework are available on the [DFO Aquaculture Science Environmental Risk Assessment Initiative webpage](#).

All risk assessments conducted under the Initiative are science-based, do not include socio-economic considerations and are not cost-benefit or risk-benefit analyses.

## **MANAGEMENT PROTECTION GOALS**

In accordance with the recommendations pertaining to aquaculture and fish health in the 2012 final report of the Commission of Inquiry into the Decline of Sockeye Salmon in the Fraser River (Cohen, 2012a), the valued ecosystem component in this risk assessment is the Fraser River Sockeye Salmon and the management protection goals are to preserve the abundance and diversity of the Fraser River Sockeye Salmon.

## **PROBLEM FORMULATION**

### **Hazard identification**

In this risk assessment, the hazard is piscine orthoreovirus (PRV) attributable to Atlantic Salmon farms in the Discovery Islands area. Given that PRV1 is the only genogroup detected in North America to date (Polinski and Garver, *in preparation*), is it the genogroup considered in this risk assessment. All mentions of PRV in this document refer to PRV1 unless specified otherwise.

### **Hazard characterisation**

Polinski and Garver (*in preparation*) summarized the relevant characteristics of PRV and of putatively associated pathologies (e.g., pathogen distribution, virulence, survival in the environment, susceptible species, shedding rates in Atlantic Salmon, virulence in Pacific salmon) and identified knowledge gaps relevant to this risk assessment.

Polinski and Garver (*in preparation*) also included a review of the occurrence of PRV and cardiopathies on Atlantic Salmon farms in BC. Additional details specific to Atlantic Salmon farms located in the Discovery Islands area are included in this risk assessment.

### **Scope**

This assessment aims to determine the risk under current farm practices, including regulatory requirements and voluntary practices as described in Wade (2017). It focuses on the risk attributable to Atlantic Salmon farms in the Discovery Islands area (Salmonid Fish Health Zone 3-2) and in close proximity (three farms in Zone 3-3 to the northwest of Zone 3-2) (refer to Figure 1 and Table 1) and includes the same 18 farms as in Mimeault et al. (2017).

Although 18 farms are included, it is worth noting that from December 2010 to February 2016, the number of stocked Atlantic Salmon farms ranged between 3 and 18, with an average of eight farms in any given month (Mimeault et al., 2017).

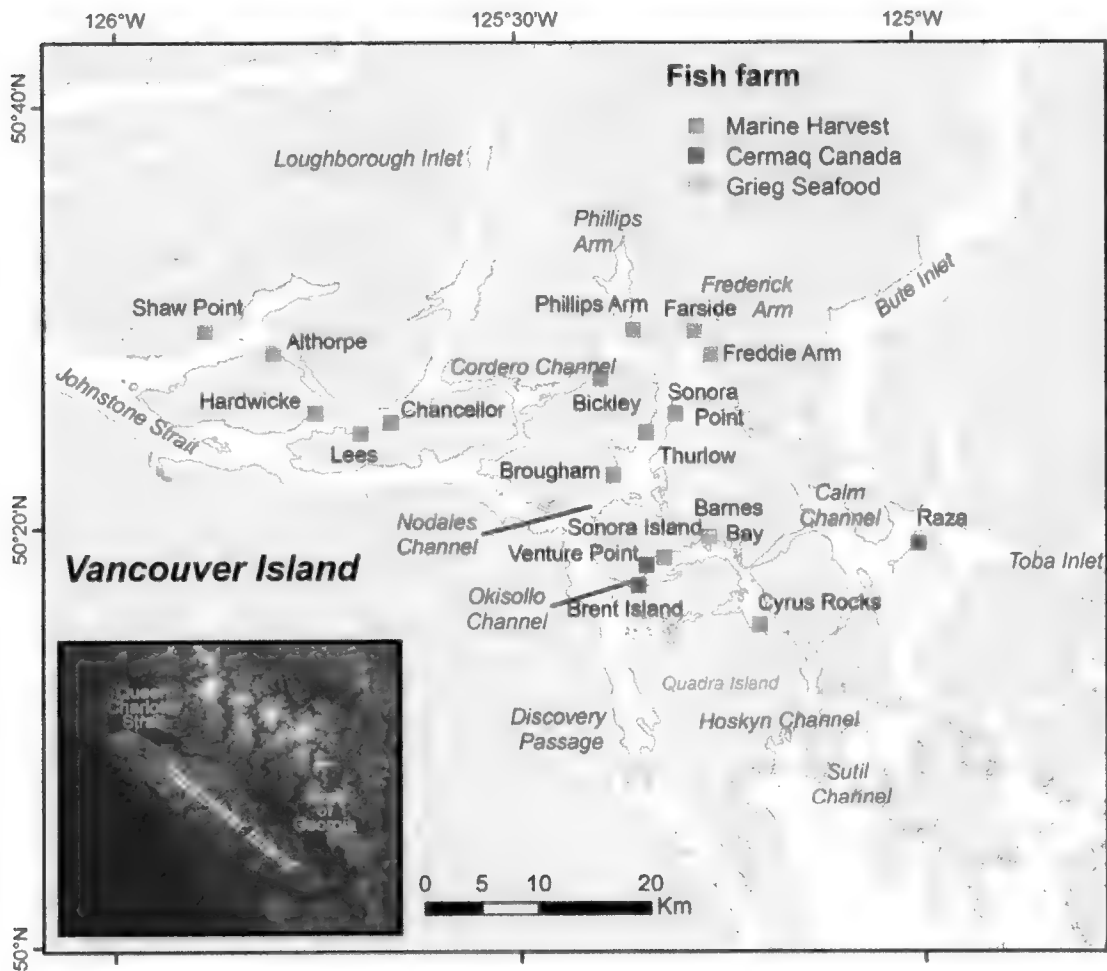


Figure 1. Locations of Atlantic Salmon farms in the Discovery Islands area (Zone 3-2 and three farms in Zone 3-3) included in this risk assessment. Symbol size for fish farms is not to scale. Different colours represent different companies operating the farms as identified in the legend. The insert illustrates the location of the Discovery Islands area in BC. Adapted from Mimeault et al. (2017).

79 *Table 1. List of the 18 Atlantic Salmon farms included in the risk assessment.*

Company	Farm	Salmonid Fish Health Zone
Cermaq Canada	Brent Island	3-2
	Raza	3-2
	Venture	3-2
Grieg Seafood	Barnes	3-2
Marine Harvest Canada	Althorpe	3-3
	Bickley	3-2
	Brougham	3-2
	Chancellor	3-2
	Cyrus Rocks	3-2
	Farside	3-2
	Freddie Arm	3-2
	Hardwicke	3-3
	Lees	3-2
	Phillips Arm	3-2
	Shaw Point	3-3
	Sonora Point	3-2
	Sonora/Okisollo	3-2
	Thurlow	3-2

80 This risk assessment focuses on the potential direct impacts of PRV attributable to Atlantic  
81 Salmon farms in the Discovery Islands area on Fraser River Sockeye Salmon abundance and  
82 diversity. Potential indirect impacts to Fraser River Sockeye Salmon through complex  
83 ecosystem processes resulting from infection of other susceptible Pacific salmon species are  
84 not considered.

### 85 **Risk question**

86 What is the risk to Fraser River Sockeye Salmon abundance and diversity due to the transfer of  
87 PRV from Atlantic Salmon farms located in the Discovery Islands area under current farm  
88 practices?

### 89 **Methodology**

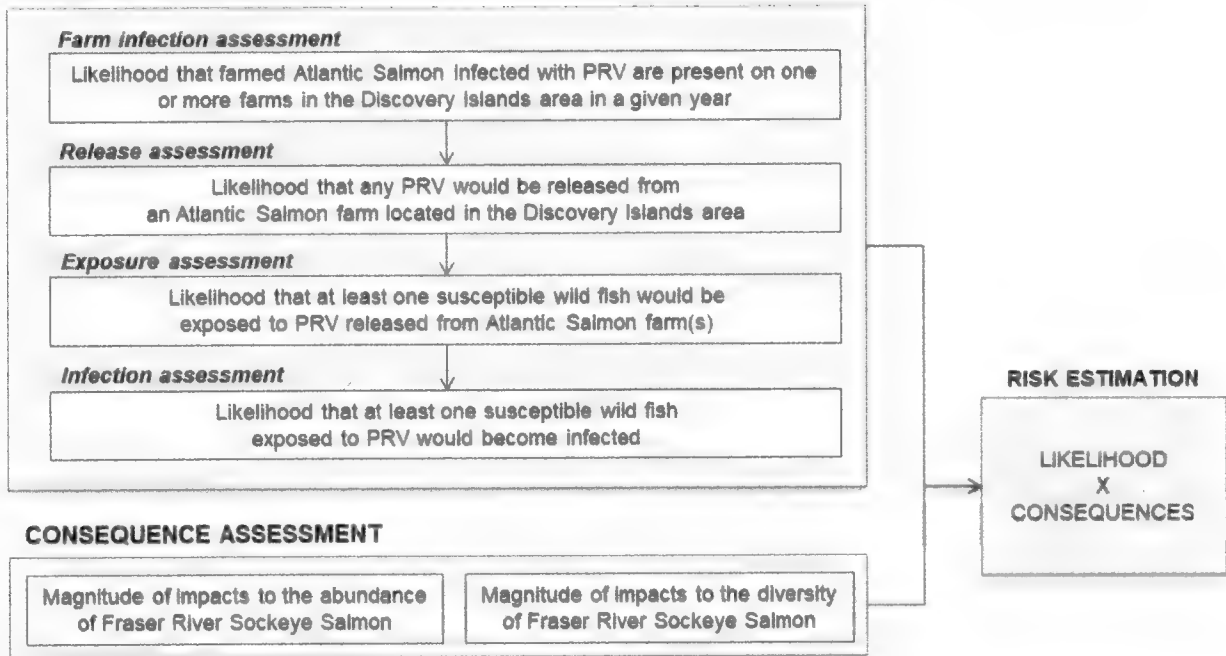
90 The methodology is based on Mimeault et al. (2017) which was adapted from the DFO  
91 Guidelines for Assessing the Biological Risk of Aquatic Invasive Species in Canada (Mandrak et  
92 al., 2012), the World Organization for Animal Health (OIE) Import Risk Analysis (OIE, 2010),  
93 recommendations for risk assessments in coastal aquaculture (GESAMP, 2008) and the Food  
94 and Agriculture Organization guidelines on understanding and applying risk analysis in  
95 aquaculture (FAO, 2008).

### 96 **Conceptual model**

97 The conceptual model (Figure 2) is adapted from Mimeault et al. (2017) in which the likelihood  
98 of an event to take place and its potential magnitude of consequences are combined into a  
99 predefined risk matrix to estimate the risk. The likelihood is assessed in four consecutive steps  
100 namely: a farm infection assessment; a release assessment; an exposure assessment; and an  
101 infection assessment. The consequence assessment determines the potential magnitude of

102 impacts of PRV infection attributable to Atlantic Salmon farms in the Discovery Islands area on  
103 the abundance and diversity of Fraser River Sockeye Salmon.

#### LIKELIHOOD ASSESSMENT



104

105 *Figure 2. Conceptual model to assess the risks to Fraser River Sockeye Salmon resulting from piscine orthoreovirus attributable to Atlantic Salmon farms located in the Discovery Islands area, BC. Adapted from Mimeault et al. (2017).*

106

#### 108 Terminology

109 The categories and definitions used to rank likelihood (Table 2), consequences to abundance  
110 (Table 3), consequences to diversity (Table 4), uncertainty for data and information (Table 5)  
111 and uncertainty for fish health management (Table 6) were adapted from Mimeault et al. (2017).

112 *Table 2. Categories and definitions used to describe the likelihood of an event over a period of a year.*  
113 *"Extremely unlikely" is the lowest likelihood and "extremely likely" is the highest likelihood. Adapted from*  
114 *Mimeault et al. (2017).*

Categories	Definitions
Extremely unlikely	Event has little to no chance to occur
Very unlikely	Event could occur rarely
Unlikely	Event could occur occasionally
Likely	Event will usually occur
Very likely	Event will occur in most instances
Extremely likely	Event will occur/is expected to occur

115

PRV risk assessment

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116 Table 3. Categories and definitions used to describe the potential consequences to the abundance of  
117 Fraser River Sockeye Salmon. Adapted from Mimeault et al. (2017).

Categories	Definitions
Negligible	0 to 1% reduction in the number of returning Fraser River Sockeye Salmon
Minor	> 1 to 5% reduction in the number of returning Fraser River Sockeye Salmon
Moderate	> 5 to 10% reduction in the number of returning Fraser River Sockeye Salmon
Major	> 10 to 25% reduction in the number of returning Fraser River Sockeye Salmon
Severe	> 25 to 50% reduction in the number of returning Fraser River Sockeye Salmon
Extreme	> 50% reduction in the number of returning Fraser River Sockeye Salmon

118 Table 4. Categories and definitions used to describe the potential consequences to the diversity of Fraser  
119 River Sockeye Salmon. CU: Conservation Unit. Adapted from Mimeault et al. (2017).

Categories	Definitions
Negligible	0 to 1% change in abundance over a generation and no loss of Fraser River Sockeye Salmon CUs over a generation
Minor	> 1 to 10% reduction in abundance in some CUs that would not result in the loss of a Fraser River Sockeye Salmon CU over a generation
Moderate	> 1 to 10% reduction in abundance in most conservation units that would not result in the loss of a Fraser River Sockeye Salmon CU over a generation; OR > 10 to 25% reduction in abundance in one or more CUs that would not result in the loss of a Fraser River Sockeye Salmon CU over a generation
Major	> 25% reduction in abundance in one or more CUs that would not result in the loss of a Fraser River Sockeye Salmon CU over a generation
Severe	Reduction in abundance that would result in the loss of a Fraser River Sockeye Salmon CU over a generation
Extreme	Reduction in abundance that would result in the loss of more than one Fraser River Sockeye Salmon CU over a generation

120 Table 5. Categories and definitions used to describe the level of uncertainty associated with data and  
121 information. Taken from Mimeault et al. (2017).

Categories	Definitions
High uncertainty	<ul style="list-style-type: none"> <li>No or insufficient data</li> <li>Available data are of poor quality</li> <li>Very high intrinsic variability</li> <li>Experts' conclusions vary considerably</li> </ul>
Reasonable uncertainty	<ul style="list-style-type: none"> <li>Limited, incomplete, or only surrogate data are available</li> <li>Available data can only be reported with significant caveats</li> <li>Significant intrinsic variability</li> <li>Experts and/or models come to different conclusions</li> </ul>
Reasonable certainty	<ul style="list-style-type: none"> <li>Available data are abundant, but not comprehensive</li> <li>Available data are robust</li> <li>Low intrinsic variability</li> <li>Experts and/or models mostly agree</li> </ul>
High certainty	<ul style="list-style-type: none"> <li>Available data are abundant and comprehensive</li> <li>Available data are robust, peer-reviewed and published</li> <li>Very low intrinsic variability</li> <li>Experts and/or models agree</li> </ul>

*Table 6. Categories and definitions used to describe the level of uncertainty associated with fish health management. "Some" and "most" are respectively defined as less and more than 50% of relevant data. Taken from Mimeault et al. (2017).*

Categories	Definitions
High uncertainty	<ul style="list-style-type: none"> <li>No information collected through farm management practices, as specified in Salmonid Health Management Plans, is available</li> <li>Discrepancy between information/data obtained through farms and farm audits for all farms</li> <li>Voluntary farm practice(s)</li> <li>Expert opinion varies considerably</li> </ul>
Reasonable uncertainty	<ul style="list-style-type: none"> <li>Some information collected through farm management practices, as specified in Salmonid Health Management Plans, is available</li> <li>Discrepancy between information/data obtained through farms and farm audits for most farms</li> <li>Voluntary company practice(s)</li> <li>Experts come to different conclusions</li> </ul>
Reasonable certainty	<ul style="list-style-type: none"> <li>Most information collected through farm management practices, as specified in Salmonid Health Management Plans, is available</li> <li>Corroboration between information/data obtained through farms and farm audits for most farms</li> <li>Voluntary industry-wide practice(s) agreed through a Memorandum of Understanding or certification by a recognized third party</li> <li>Experts mostly agree</li> </ul>
High certainty	<ul style="list-style-type: none"> <li>All information collected through farm management practices, as specified in Salmonid Health Management Plans, is available</li> <li>Corroboration between information/data obtained through farms and farm audits for all farms</li> <li>Mandatory practice(s) required under legislation and certification by a recognized third party</li> <li>Experts agree</li> </ul>

## Combination rules

As described in Mimeault et al. (2017), the combination of likelihoods differs if events are dependent or independent: "An event is dependent when its outcome is affected by another event. For example, infection can only happen if exposure took place, consequently infection is dependent on exposure. Events are independent when the outcome of one event does not affect the outcome of other event(s); for example, a pathogen can be released into the environment via different unrelated pathways". Likelihoods are combined as per accepted methodologies in qualitative risk assessments adopting the lowest value (e.g., low) for dependent events and the highest value (e.g., high) for independent events (Cox, 2008; Gale et al., 2010; Cudmore et al., 2012).

Uncertainties are reported at each step of the risk assessment. Several approaches have been used for combining qualitative uncertainty rankings in risk assessments. Some authors report uncertainty for every step without combination (Peeler and Thrush, 2009; Jones et al., 2015), others adopt the highest uncertainty (Mandrak et al., 2012) while finally others adopt the highest uncertainty associated with the lowest likelihood for dependent events (Cudmore et al., 2012). In this risk assessment, uncertainties are not combined in the overall likelihood and consequence assessments to keep the emphasis on the uncertainty associated to each step.

## Risk estimation

As described in Mimeault et al. (2017), two risk matrices were developed in collaboration with DFO's Ecosystems and Oceans Sciences and Ecosystem and Fisheries Management sectors to categorize the risk estimates for the abundance (Figure 3) and diversity (Figure 4) of Fraser River Sockeye Salmon. They are aligned with relevant scale of consequences for fisheries management and policy purposes, existing policy and current management risk tolerance relevant to the risk assessments.

Likelihood	Extremely likely						
	Very likely						
	Likely						
	Unlikely						
	Very unlikely						
	Extremely unlikely						
		Negligible	Minor	Moderate	Major	Severe	Extreme
Consequences to Fraser River Sockeye Salmon abundance							

Figure 3. Risk matrix for combining the results of the assessment of the likelihood and consequences to Fraser River Sockeye Salmon abundance. Green, yellow and red, respectively, represent minimal, moderate and high risk.

Likelihood	Extremely likely						
	Very likely						
	Likely						
	Unlikely						
	Very unlikely						
	Extremely unlikely						
		Negligible	Minor	Moderate	Major	Severe	Extreme
Consequences to Fraser River Sockeye Salmon diversity							

Figure 4. Risk matrix for combining the results of the assessment of the likelihood and consequences to Fraser River Sockeye Salmon diversity. Green, yellow and red, respectively, represent minimal, moderate and high risk.

## LIKELIHOOD ASSESSMENT

The likelihood assessment consists of determining the likelihood that Fraser River Sockeye Salmon would become infected with piscine orthoreovirus (PRV) attributable to Atlantic Salmon farms located in the Discovery Islands area.

Each step of the likelihood assessment assumes that current management practices on Atlantic Salmon farms are followed and will be maintained.

## FARM INFECTION ASSESSMENT

### Question

In a given year, what is the likelihood that farmed Atlantic Salmon infected with PRV are present on one or more farms in the Discovery Islands area?



## Considerations

Considerations include evidence of Atlantic Salmon susceptibility to PRV; regulatory requirements; industry practices; PRV prevalence in hatcheries; and evidence of PRV on Atlantic Salmon farms in the Discovery Islands area.

## Atlantic Salmon susceptibility to PRV infection

PRV genetic material has been detected in Atlantic Salmon in several countries (Palacios et al., 2010; Kibenge et al., 2013; Marty et al., 2015; Adamek et al., 2018; Gunnarsdóttir et al., 2018; Markussen et al., 2018; Warheit, 2018).

More specifically, Atlantic Salmon infection with the PRV genetic type from Pacific Canada has been demonstrated through a cohabitation study (Garver et al., 2016a) and PRV has been reported on Atlantic Salmon farms in BC (Marty et al., 2015; Di Cicco et al., 2017; Nekouei et al., 2018; Laurin et al., 2019) supporting Atlantic Salmon susceptibility to PRV.

## Regulatory requirements

### *Licensing and biosecurity*

DFO has had the primary responsibility for the regulation and management of aquaculture in BC since December 2010 through the Pacific Aquaculture Regulations (PAR) developed under the Fisheries Act. DFO is therefore responsible for issuing aquaculture licenses for marine finfish, shellfish and freshwater operations in BC.

Each farm operating in BC requires a Finfish Aquaculture Licence under the PAR which includes the requirement for a Salmonid Health Management Plan (SHMP) and accompanying proprietary Standard Operating Procedures (SOPs) (DFO, 2015). The SHMP outlines the health concepts and required elements associated with a finfish aquaculture licence, while accompanying SOPs detail the procedures to address specific concepts of the SHMP including monitoring fish health and diseases (DFO, 2015; Wade, 2017).

The SHMP includes requirements related to "Keeping Pathogens Out" (section 2.5 of the SHMP) (DFO, 2015) including that particular care be taken to avoid undue fish stress and transmission of pathogens and also requires a licence by the Introductions and Transfer Committee in advance of any fish transfers (DFO, 2015).

### *Fish Health Audit and Surveillance Program*

Samples from recently dead fish are collected through the Fish Health Audit and Surveillance Program (audit program) to audit the routine monitoring and reporting of diseases by the farms (Wade, 2017). Moribund fish can also be sampled (I. Keith, DFO, 103-2435 Mansfield Drive, Courtenay, BC V9N 2M2, pers. comm., 2018). DFO aims to audit 30 randomly selected farms per quarter or 120 farms per year (Wade, 2017).

During an audit, a maximum of 30 fresh fish are selected for histopathology, bacteriology and molecular diagnostics/virology, although in most circumstances eight fresh fish are sampled (Wade, 2017). PRV is not included in the molecular diagnostics completed on audit samples.

### *Introduction and Transfer Committee*

DFO grants Introduction and Transfer licenses under Section 56 of the Fishery (General) Regulations. The Introductions and Transfers Committee (ITC) assesses the health, genetic and ecological impacts that could occur through the transfer of fish into and within the Province. A Section 56 introductions and transfers licence is required for all movements of salmon between licensed aquaculture facilities (DFO, 2018b). For the aquaculture industry, the committee

assesses the health of fish to be transferred which includes the diseases and causative agents of regional, national or international concern as listed in Appendix III<sup>1</sup> of the Marine Finfish Aquaculture Licence under the Fisheries Act, along with any other concern that may arise during the assessment.

For every aquaculture related transfer application, fish health reports and husbandry records are examined by Aquaculture Management Division staff prior to transfer. If any clinical signs of diseases are seen, or there are any other concerns, the committee can either recommend that the transfer should not happen, or they can work with the applicant to ensure the transfer is carried out in a safe manner. Currently, there are no requirements to test for PRV prior to the transfer of fish into marine net pens or between sites (M. Higgins, Fisheries and Oceans Canada, pers. comm., 2018).

### Industry practices

Companies rearing Atlantic Salmon on marine sites in the Discovery Islands area are Cermaq Canada, Grieg Seafood and Marine Harvest Canada.

#### *Movement of live fish*

Between January 2013 and December 2017, Atlantic Salmon have been present on farms in the Discovery Islands area throughout the year (Appendix A, Figure 6). The duration of farmed Atlantic Salmon production cycles in the Discovery Islands area over the same period ranged between 12 and 23 months (average=17 months, n=27 cycles) from the beginning of stocking to the end of harvesting periods.

In the Discovery Islands area, smolts are not transferred directly from freshwater hatcheries to marine sites due to the risk of infection from *Kudoa* sp., a parasite of marine fishes (Wade, 2017) with the exception of Raza where *Kudoa* sp. has not been an issue (Danielle New, Cermaq Canada, 203-919 Island Highway, Campbell River, BC, Canada V9W 2C2, pers. comm., 2018).

Fish transfers to marine grow-out sites in the Discovery Islands area occurred every months of the year, with most of them in May and June (Appendix A, Figure 7). Fish reared in this area can previously spend between 2 to 14 months (average=7 months, n=23 cycles) on a marine nursery site before being transferred to a grow-out site.

#### *Surveillance and testing*

Every stocked marine production site is monitored daily by on-site trained staff for syndromic surveillance during which mortalities are removed and classified. Staff alerts the veterinarian if there are any concerns. Additionally, routine health checks are conducted regularly by all companies during which fresh mortalities and/or silvers are examined for signs of diseases or abnormal conditions and sampled for pathogen screening on an as needed basis based on syndromic surveillance, site history, environmental conditions and professional judgement of the

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<sup>1</sup> In 2018, diseases of regional, national or international concern listed in the Marine Finfish Aquaculture Licence under the Fisheries Act are Infectious Hematopoietic Necrosis (IHN) and infectious hematopoietic necrosis virus; Infectious Pancreatic Necrosis (IPN) and infectious pancreatic necrosis virus; Viral Hemorrhagic Septicemia (VHS) and viral hemorrhagic septicemia virus; Infectious Salmon Anemia (ISA) and infectious salmon anemia virus; *Oncorhynchus masou* Virus Disease (OMV) and *Oncorhynchus masou* virus; Whirling Disease and *Myxobolus cerebralis*; Cold Water Vibriosis and *Vibrio salmonicida*; and any other filterable replicating agent causing cytopathic effects in cell lines specified by the Minister or is causative of identifiable clinical disease in fish.

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veterinarian and fish health team. The frequency of routine health checks and sampling for pathogen screening varies among companies as described below.

In addition to daily monitoring, every Cermaq Canada stocked marine production site is visited by fish health staff or the veterinarian a minimum of once every two weeks to confirm on-site mortality classification and to sample up to five moribund or fresh mortalities with no obvious cause of death (e.g., non-performing, algae, handling, low oxygen, matures, deformities) (Cermaq Canada, pers. comm., 2018).

In addition to daily monitoring, every Grieg Seafood stocked marine production site is visited at least once every quarter by the fish health staff and/or veterinarian where at least five silvers are sampled for bacteriology, histology and PCR testing (Grieg Seafood, pers. comm., 2018).

In addition to daily monitoring, every Marine Harvest Canada stocked production site is visited at least once a month by fish health staff or the veterinarian and at least once every quarter by the veterinarian. Fresh mortalities and/or silver samples may be collected for pathogen screening based on syndromic surveillance, site history, environmental conditions and professional judgement of the veterinarian and the fish health team (Marine Harvest Canada, pers. comm., 2018).

*Vaccination and treatment*

There is no commercial vaccine available for PRV nor are there treatments available for PRV-infected Atlantic Salmon. There is no data to suggest that PRV adversely affects aquaculture production of salmon in BC (Polinski and Garver, *in preparation*).

**PRV prevalence in Atlantic Salmon hatcheries**

Industry conducts sampling for PRV screening including prior to fish transfers to marine sites. Table 7 presents last PRV screening results of Atlantic Salmon sampled in BC hatcheries prior to transfer to a marine site, either directly or indirectly into the Discovery Islands area. This represents a proportion of the overall hatchery PRV screening that the industry conducts.

Between 2013 and 2018, PRV has been detected in hatcheries in all years, with percent PRV positive sampled fish ranging between 0.2 to 72.5%. The trends observed show an increase in the number of samples collected during this period and a decrease in the percentage of PRV positive sampled fish.

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*Table 7. PRV screening conducted between 2013 and 2018 in Atlantic Salmon in hatcheries prior to direct or indirect transfer to marine sites in the Discovery Islands area, BC. Results only include last sampling events prior to transfer. Source: Data provided by the industry in January 2019.*

Year	Number of fish screened for PRV *	Number of PRV positive fish	Percent PRV positive fish
2013	48	20	41.7
2014	40	29	72.5
2015	110	29	26.4
2016	189	21	11.1
2017	370	3	0.8
2018	584	1	0.2

\* Three sampling events (two in 2015 and one in 2016), out of a total of 42, had unspecified number of fish for which 25 fish per sampling event were assumed

**PRV prevalence on Atlantic Salmon farms in BC**

Several studies have reported PRV on Atlantic Salmon farms in BC (Marty et al., 2015; Di Cicco et al., 2017; Laurin et al., 2019).

Marty et al. 2015 reported 95% (35/37) of archived samples of farmed Atlantic Salmon collected between 2000 and 2008 from DFO management areas 7, 12, 13 and 18 (respectively Prince and Hunter Islands; Northern Johnstone Strait; Quadra and Cortes Islands; and Mayne Island, Saanich) and 100% (20/20) of Atlantic Salmon sampled in 2013 from a marine rearing site in the Northern Johnstone Strait approximately one month after transfer from a hatchery, to be PRV positive.

Di Cicco et al. (2017) reported 19% (8/42) of Atlantic Salmon sampled on a farm in BC in 2013 about three to four months after seawater transfer and 100% (43/43) of those sampled after five to six months in seawater, to be PRV positive.

Laurin et al. (2019) reported 67% (448/668) of all recently dead and moribund Atlantic Salmon sampled through the audit program between April 2011 and December 2013 on farms across BC to be PRV positive; a proportion that varied approximately from 40% to nearly 90% among different fish health zones in BC. Time-at-sea was a significant predictor for PRV detection in Atlantic Salmon with the highest odds of detecting the virus reported 12 to 18 months after transfer to seawater (Laurin et al., 2019).

In on-going research examining PRV prevalence on thirteen Atlantic Salmon farms in BC, including in the Discovery Islands area, all sites became PRV positive with a general onset between approximately 100 to 200 days after seawater entry and 100% of samples (132/132) collected from fish at sea for more than 296 days were PRV positive (Polinski and Garver, unpublished data reported in Polinski and Garver (*in preparation*)).

Although the above studies are not limited to Atlantic Salmon farms in the Discovery Islands area and the average proportion of PRV positive recently dead and moribund farmed Atlantic Salmon was reported to vary among fish health zones (Laurin et al., 2019), overall PRV is ubiquitous, highly prevalent and persistent on Atlantic Salmon farms in BC.

PRV screening results provided by the industry to support this risk assessment also indicate that most fish become infected with the virus at some point in the marine grow-out phase.

## Assumptions

- Positive detection of the pathogen is evidence of infection; and
- Results from research studies throughout all zones are representative of the Discovery Islands area.

## Likelihood of farm infection

Table 8 presents the main factors contributing to and limiting the likelihood of a PRV infection occurring on an Atlantic Salmon farm in the Discovery Islands area. Those factors were used to determine the likelihood and uncertainty rankings based on definitions in tables 2, 5 and 6.

*Table 8. Factors contributing to and limiting piscine orthoreovirus infection pressure from Atlantic salmon farms in the Discovery Islands area under the current farm practices.*

Contributing factors	Limiting factors
<ul style="list-style-type: none"> <li>• Atlantic Salmon are susceptible to PRV;</li> <li>• All Atlantic Salmon farms in the Discovery Islands area are anticipated to become infected with PRV within 100-200 days post-seawater transfer;</li> <li>• Independent of farm location or season of transfer to seawater, Atlantic Salmon farms become infected with PRV and can reach 100%;</li> <li>• In the Discovery Islands area, except for one site, smolts are transferred from other marine rearing sites;</li> <li>• Smolts may be held from 2 to 14 months in marine nursery sites before transfer to Discovery Islands area; and</li> <li>• Current regulatory requirements for an aquaculture-related BC introduction and transfers licence are related to clinical signs of disease and/or the detection of the causative agents listed in Appendix III of the Marine Finfish Aquaculture Licence under the Fisheries Act which does not include PRV.</li> </ul>	<ul style="list-style-type: none"> <li>• Hatchery-origin infection is mitigated through egg disinfection, a requirement of the SHMP and other biosecurity practices.</li> </ul>

It was concluded that, in a given year, the likelihood that farmed Atlantic Salmon infected with PRV are present on one or more Atlantic Salmon farms in the Discovery Islands area is **extremely likely** under the current farm practices given the evidence of PRV infection on Atlantic Salmon farms following seawater transfer. This conclusion was made with **high certainty** given abundant and robust data demonstrating PRV infections on Atlantic Salmon farms in BC.

324 **RELEASE ASSESSMENT**

325 **Question**

326 Assuming that Atlantic Salmon infected with PRV are present, what is the likelihood that any  
327 PRV would be released from an Atlantic Salmon farm located in the Discovery Islands area into  
328 an environment accessible to wild fish populations?

329 **Considerations**

330 Considerations include Atlantic Salmon rearing conditions in the Discovery Islands area;  
331 shedding of PRV from infected fish; and fish health management practices.

332 **Atlantic Salmon rearing methods**

333 Atlantic Salmon reared on marine sites in the Discovery Islands area are contained in net pens.  
334 Under such conditions, water flows freely through the cages and there are no barriers to  
335 pathogen exchanges between the net pens and the environment (Johansen et al., 2011).

336 **Shedding of PRV from infected fish**

337 Polinski and Garver (*in preparation*) reviewed the state of knowledge related to shedding in  
338 PRV-infected fish. Given evidence of horizontal transmission during cohabitation study (Garver  
339 et al., 2016a), PRV infected salmon are considered to be a source of the virus (Polinski and  
340 Garver, *in preparation*). PRV has been detected in faecal contents of Atlantic Salmon  
341 challenged through injections or anal intubation with a PRV inoculum originating from  
342 Norwegian field heart and skeletal muscle inflammation (HSMI) outbreak (Hauge et al., 2016).  
343 The above studies provide evidence that PRV-infected fish can shed the virus into the  
344 surrounding environment.

345 To this date, the rate of shedding from PRV-infected Atlantic Salmon (or other salmonids) has  
346 not been quantified (Polinski and Garver, *in preparation*). However, based on cohabitation  
347 studies (Garver et al., 2016a; Polinski et al., *in press*), it is hypothesized that horizontal  
348 transmission primarily occurs between 3 to 15 weeks following infection, after which the  
349 potential for natural shedding becomes severely reduced despite persistence of infection  
350 (Polinski and Garver, *in preparation*).

351 **Fish health management practices**

352 All licence holders must comply with the Health Management Plan which includes biosecurity  
353 measures to maintain fish health, prevent pathogen entry and limit the spread of diseases on  
354 farm (DFO, 2015).

355 The Salmonid Health Management Plan (SHMP) requires procedures for collecting,  
356 categorizing, recording, storing and disposing of fish carcasses (DFO, 2015). More specifically,  
357 procedures must be in place for the regular removal of carcasses to storage containers; the  
358 reporting of mortality by category to DFO; a secure location of stored carcasses until transfer to  
359 land-based facilities; to prevent contents from leaking into the receiving waters; the secure  
360 transfer of stored carcasses to land-based facilities; and sanitization methods for storage  
361 containers, equipment and other handling facilities or vessels (DFO, 2015). The SHMP also  
362 requires a SOP for fish disease outbreaks or emergency, where an outbreak is defined as an  
363 "unexpected occurrence of mortality or disease" (DFO, 2015).

364 Beyond indicating if a SOP is required, DFO does not prescribe how elements of the SHMP  
365 should be achieved. It is therefore up to the company to address the concepts to the satisfaction

of the DFO's fish health veterinarian (Wade, 2017). Consequently, it is assumed that for companies with a valid finfish aquaculture licence, the SOPs submitted are in compliance with the conditions of licence and approved by the DFO veterinarian (Wade, 2017).

Protocols are in place for handling and storing dead fish; for labeling, cleaning, disinfecting and storing gear used to handle dead fish; to restrict visitors who must obtain permission prior to arriving on site; to control on-site visitors through the use of signage, footbaths and site specific protective clothing; net washing procedures, not sharing equipment when possible, cleaning and disinfecting equipment after use and dry storing in proper locations; for cleaning, disinfecting and transferring large and submerged equipment among sites; and biosecurity measures to control vessel movement (Wade, 2017).

Compliance with the above elements is determined through the audit program. On average, less than one deficiency has been reported per audit on Atlantic Salmon farms in BC between 2011 and 2017 (Appendix B, Table 15). Most deficiencies reported in this period were related to sea lice protocols and sea lice records; carcass retrieval protocol or record keeping that requires improvement; mooring signage needing improvement; and transfer records not being complete.

### Assumptions

- Atlantic Salmon infected with PRV are present on at least one farm; and
- Biocontainment measures are effective against PRV (e.g., Virkon footbaths, etc.).

### Likelihood of release

Table 9 presents the main factors contributing to and limiting the likelihood that PRV would be released from an infected Atlantic Salmon farm in the Discovery Islands area. These factors were used to determine the likelihood and uncertainty rankings based on definitions in Tables 2, 5 and 6.

*Table 9. Factors contributing to and limiting the likelihood that piscine orthoreovirus will be released from infected and/or diseased Atlantic Salmon on farms in the Discovery Islands area under the current farm practices.*

Contributing factors	Limiting factors
<ul style="list-style-type: none"> <li>PRV-infected Atlantic Salmon can shed the virus into the surrounding environment; and</li> <li>Atlantic Salmon in the Discovery Islands area are reared in net pens allowing pathogens, including PRV, to be released from the farms to the surrounding environment.</li> </ul>	<ul style="list-style-type: none"> <li>Protocols are in place for handling and storing dead fish; for labeling, cleaning, disinfecting and storing gear used to handle dead fish;</li> <li>Protocols are in place to restrict visitors who must obtain permission prior to arriving on site and to control on-site visitors through the use of signage, footbaths and site specific protective clothing;</li> <li>Protocols are in place to minimize predators and wildlife access;</li> <li>Protocols are in place for net washing procedures, not sharing equipment when possible, cleaning and disinfecting equipment after use and dry storing in proper locations;</li> </ul>



	<ul style="list-style-type: none"> <li>• Protocols are in place for cleaning, disinfecting and transferring large and submerged equipment among sites;</li> <li>• Biosecurity measures are in place to control vessel movement; and</li> <li>• Low levels of operational deficiencies that could affect fish health have been reported on Atlantic Salmon farms in the Discovery Islands area.</li> </ul>
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392 Two pathways were considered in the release assessment: (1) infected farmed Atlantic Salmon  
393 and (2) mechanical vectors and fomites.

#### 394 **Release through infected farmed Atlantic Salmon**

395 It was concluded that the likelihood that PRV would be released from an Atlantic Salmon farm  
396 located in the Discovery Islands area into an environment accessible to Fraser River Sockeye  
397 Salmon through infected farmed Atlantic Salmon is **extremely likely** under the current farm  
398 practices given rearing of Atlantic Salmon in net pens and evidence that infected Atlantic  
399 Salmon can shed the virus. This conclusion was made with **high certainty** based on robust  
400 published laboratory studies on horizontal transfer and infection through cohabitation studies.

#### 401 **Release through vectors and fomites**

402 It was concluded that the likelihood that PRV would be released from an Atlantic Salmon farm  
403 located in the Discovery Islands area into an environment accessible to wild fish populations  
404 through vectors or fomites is **unlikely** under the current farm practices. This conclusion was  
405 made with **reasonable uncertainty** given relevant biosecurity practices are part of licence  
406 requirements and low levels of operational deficiencies that could affect fish health on Atlantic  
407 Salmon farms in the Discovery Islands area but also given the use of proxy data and  
408 assumption that biocontainment practices are effective against PRV.

#### 409 **Overall likelihood of release**

410 The overall likelihood of release was obtained by adopting the highest likelihood of the release  
411 pathways. It is therefore **extremely likely** that PRV would be released from an Atlantic Salmon  
412 farm should it become infected.

### 413 **EXPOSURE ASSESSMENT**

#### 414 **Question**

415 Assuming that PRV has been released from at least one Atlantic Salmon farm in the Discovery  
416 Islands area, what is the likelihood that at least one Fraser River Sockeye Salmon would be  
417 exposed to PRV in a given year?

#### 418 **Considerations**

419 The exposure assessment consists of determining the spatial and temporal concurrence of the  
420 released pathogen and susceptible species (Taranger et al., 2014).

421 Considerations include size and volume of Atlantic Salmon farms; occurrence of Fraser River  
422 Sockeye Salmon in the Discovery Islands area; timing of PRV on Atlantic Salmon farms;

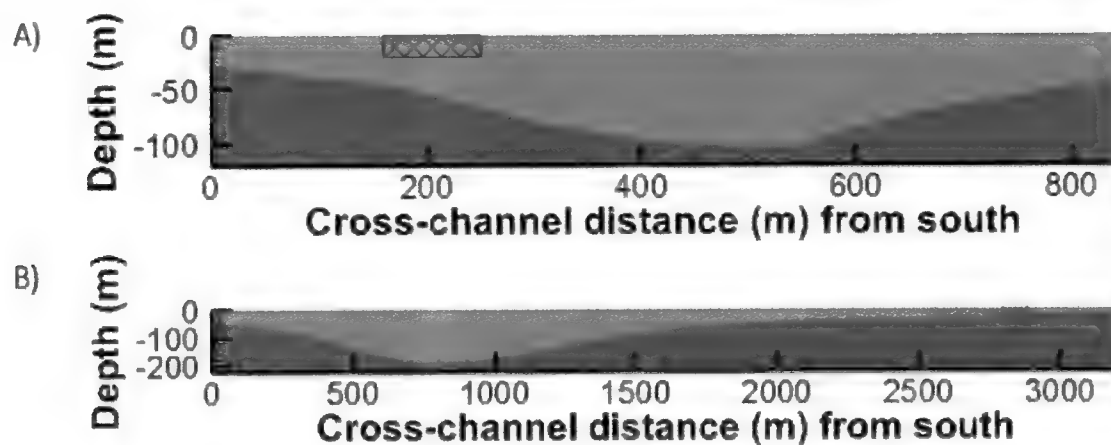


423 survival of PRV in the marine environment; and concurrence of PRV and Fraser River Sockeye  
424 Salmon.

#### 425 **Size and volume of Atlantic Salmon farms**

426 The likelihood of Fraser River Sockeye Salmon to encounter Atlantic Salmon farms on their  
427 migration routes should take into account the relative size and volume of farms in the area and  
428 within channels.

429 Atlantic Salmon farms in the Discovery Islands area occupy an extremely small area (0.007%)  
430 and volume (0.0008%) of the overall region (Mimeault et al., 2017). Additionally, considering  
431 that channel width in the Discovery Islands area varies between approximately 850 and 3,200  
432 meters (Mimeault et al., 2017), a farm with dimension of 100 m by 100 m by 20 m depth would  
433 span over approximately 3 to 12% of the width of the channel (Figure 5).



434  
435 *Figure 5. Cross sections of channels at (A) Brent and (B) Shaw farms located in respectively the*  
436 *narrowest and widest channel with Atlantic Salmon farms in the Discovery Islands area. Cross-hatched*  
437 *boxes show the cross-channel projection of the net-pens of the farms depicted at scale, i.e., what fish*  
438 *swimming along-channel would encounter. Note the difference in the ranges on the axes to maintain*  
439 *constant ratio (one:one) between the x and y axes in each cross section. Adapted from Mimeault et al.*  
440 *(2017).*

#### 441 **Fraser River Sockeye Salmon in Discovery Islands area**

##### 442 *Out-migrating juveniles*

443 Juvenile Fraser River Sockeye Salmon migrate through the Discovery Islands area every year  
444 from mid-May to mid-July (reviewed in Grant et al., 2018). The total number of juveniles out-  
445 migrating from the Fraser River is unknown (Grant et al., 2018). The only estimate of  
446 abundance is limited to stocks from Chilko Lake (Grant et al., 2018) based on smolts  
447 enumerated at a counting fence located at the outlet of the lake. Between 1953 and 2007,  
448 annual estimates ranged between 1.6 to 77 million (average: 20 million) (Grant et al., 2018).

Knowledge of juvenile marine out-migration routes through the Discovery Islands area is limited, however, based on 2016 and 2017 telemetry-based results, 37 to 73% (average of 55%<sup>2</sup>) of Chilko Lake Sockeye Salmon migrated east of Quadra Island (Rechisky et al., 2018).

#### *Returning adults*

Sockeye Salmon return to the Fraser River either through the northern route (Johnstone Strait) or the southern route (Strait of Juan de Fuca) (reviewed in Grant et al., 2018). Between 1980 and 2014, the total adult returns of Fraser River Sockeye Salmon ranged from 2 to 28 million, with an annual average of 9.6 million (Grant et al., 2018).

The proportion of Sockeye Salmon that migrate through the northern route, referred to as the northern diversion rate, is highly variable. Based on data provided by the Pacific Salmon Commission, the northern diversion rate between 1980 and 2015 ranged between 10% in 2008 and 96% in 2014 (average of 52%). Returning adult Fraser River Sockeye Salmon migrate through the Discovery Islands area from late-June to early-October (reviewed in Grant et al., 2018).

#### **Timing of PRV on Atlantic Salmon farms**

PRV has been reported on Atlantic Salmon farms in BC (Marty et al., 2015; Di Cicco et al., 2017; Laurin et al., 2019). Refer to Farm Infection Assessment section for more details on prevalence. Of relevance to the exposure assessment is the timing of PRV detections on Atlantic Salmon farms in the Discovery Islands area.

PRV was detected in Atlantic Salmon sampled in the month of April 2013 on a marine rearing site in BC (Marty et al., 2015); in the months of August through November 2013 and January 2014 on a marine rearing site in BC (Di Cicco et al., 2017); and between April 2011 and December 2013 through the DFO Regulatory Fish Health Audit Program on marine sites in BC, including in the Discovery Islands area (Laurin et al., 2019). Finally, on-going investigations examining PRV prevalence on thirteen Atlantic Salmon farms in BC detected PRV infections on Atlantic Salmon farms in the Discovery Islands area throughout the year.

Given that fish are transferred to marine rearing sites in the Discovery Islands area throughout the year (Appendix A, Figure 7), sites in this area could theoretically become positive throughout the year. While the sample sizes used are small, the results have been consistent throughout farms sampled in BC.

Overall, PRV has been reported on at least one Atlantic Salmon farm in the Discovery Islands area in all months of the year.

#### **PRV survival in the marine environment**

No studies have been conducted on the survival of PRV in the environment and suitable surrogate data are not available (Polinski and Garver, *in preparation*). However, given that waterborne transmission of PRV has been demonstrated in seawater (Garver et al., 2016a; Polinski et al., *in press*), and PRV being free of an envelope, it can be presumed that it maintains a minimum capacity to survive in water even if the duration of survival and infectivity in seawater are unknown (Polinski and Garver, *in preparation*).

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<sup>2</sup> Average of tagged fish reported to migrate east of Quadra Islands (37% of age-1 Chilko Lake Sockeye Salmon in 2016, 73% of age-1 Chilko Lake Sockeye Salmon in 2017 and 54% of age-2 Chilko Lake Sockeye Salmon) (Rechisky et al., 2018).

## Concurrence between Fraser River Sockeye Salmon and PRV

### Spatial

Given evidence of juvenile and adult Fraser River Sockeye Salmon migration through the Discovery Islands area and evidence of PRV on at least one Atlantic Salmon farm in the Discovery Islands area, it was concluded that there is potential spatial concurrence between Fraser River Sockeye Salmon and PRV attributable to Atlantic Salmon farms in the Discovery Islands area.

### Temporal

Table 10 summarizes evidence of temporal overlap between Fraser River Sockeye Salmon and PRV on Atlantic Salmon farms in the Discovery Islands area. Given that (1) Fraser River Sockeye Salmon are present in the Discovery Islands area between May and October; (2) Atlantic Salmon farms in the Discovery Islands area are stocked throughout the year; and (3) PRV has been reported throughout the year on Atlantic Salmon farms in the Discovery Island area, it was concluded that there is temporal concurrence between Fraser River Sockeye Salmon and PRV attributable to Atlantic Salmon farms in the Discovery Islands area.

*Table 10. Summary of evidence of temporal overlap between Fraser River Sockeye Salmon and piscine orthoreovirus on Atlantic Salmon farms in the Discovery Islands area. The "X" indicates evidence of presence of Fraser River Sockeye Salmon in a given month; letters on the first row of the table represent months of the year from January to December. Data source: Marty et al. (2015); Di Cicco et al. (2017); Grant et al. (2018); Laurin et al. (2019) and unpublished data reported in Polinski and Garver (in preparation).*

Fraser River Sockeye Salmon in the Discovery Islands area	J	F	M	A	M	J	J	A	S	O	N	D
Lake-type juvenile					X	X	X					
Returning adult						X	X	X	X	X		
Farmed Atlantic Salmon in the Discovery Islands area	J	F	M	A	M	J	J	A	S	O	N	D
Stocked net pens	X	X	X	X	X	X	X	X	X	X	X	X
Stocking events	X	X	X	X	X	X	X	X	X	X	X	X
PRV positive detections	X	X	X	X	X	X	X	X	X	X	X	X

## Assumptions

- At least one Fraser River Sockeye Salmon has been exposed to PRV released from Atlantic Salmon farms in the Discovery Islands area;
- Positive detections of PRV is evidence that the pathogen is present in sampled fish;
- PRV-infected fish are shedding the virus;
- Shedding occurs during months with evidence of infection on farms;
- Pacific salmon can use all channels in the Discovery Islands area; and

- Wild Sockeye Salmon and Sockeye Salmon produced through enhancement are not differentiated for the purpose of this risk assessment.

### Likelihood of exposure

Table 11 presents the main factors contributing to and limiting the likelihood of Fraser River Sockeye Salmon to be exposed to PRV attributable to Atlantic Salmon farm(s) in the Discovery Islands area. Those factors were used to determine the likelihood and uncertainty rankings based on definitions in Tables 2, 5 and 6.

*Table 11. Factors contributing to and limiting the likelihood that Fraser River Sockeye Salmon would be exposed to piscine orthoreovirus released from infected Atlantic Salmon farm(s) in the Discovery Islands area under the current farm practices.*

Contributing factors	Limiting factors
<ul style="list-style-type: none"> <li>• Juvenile and adult Fraser River Sockeye Salmon migrate through the Discovery Islands area every year;</li> <li>• All Atlantic Salmon farms in the Discovery Islands area are anticipated to become infected with PRV within 100-200 days post-seawater transfer; and</li> <li>• There is temporal overlap between Fraser River Sockeye Salmon migration (May through October) and the presence of PRV on Atlantic Salmon farms in the Discovery Islands area.</li> </ul>	<ul style="list-style-type: none"> <li>• Atlantic Salmon farms are not found in all channels of the Discovery Islands area; and</li> <li>• Atlantic Salmon farms occupy a very small surface area and volume of the Discovery Islands area and width of channels.</li> </ul>

Two exposure groups were assessed: (1) juvenile Fraser River Sockeye Salmon; and (2) adult Fraser River Sockeye Salmon. Waterborne exposure is considered as the most relevant exposure route for Fraser River Sockeye Salmon in the context of this risk assessment.

### Exposure of juvenile Fraser River Sockeye Salmon

It was concluded that the likelihood of at least one juvenile Fraser River Sockeye Salmon to be exposed to PRV attributable to Atlantic Salmon farms located in the Discovery Islands area through waterborne exposure is **extremely likely** under the current farm practices given the temporal overlap with reports of PRV on farms. This conclusion was made with **reasonable certainty** given abundant and robust data documenting the presence of juvenile Sockeye Salmon in the Discovery Islands area but lack of knowledge on the spatial and temporal distribution in proximity to farms and PRV survival in the marine environment.

### Exposure of adult Fraser River Sockeye Salmon

It was concluded that the likelihood of at least one adult Fraser River Sockeye Salmon to be exposed to PRV attributable to an Atlantic Salmon farm located in the Discovery Islands area through waterborne exposure is **extremely likely** under the current farm practices given the temporal overlap with reports of PRV on farms. This conclusion was made with **reasonable certainty** given abundant and robust data documenting the presence of adult Sockeye Salmon in the Discovery Islands area but lack of knowledge on the spatial and temporal distribution in proximity to farms and PRV survival in the marine environment.

## INFECTION ASSESSMENT

### Question

Assuming that at least one Fraser River Sockeye Salmon has been exposed to PRV released from Atlantic Salmon farms in the Discovery Islands area, what is the likelihood that at least one will become infected?

### Considerations

The infection assessment consists of determining the likelihood that Fraser River Sockeye Salmon will be exposed to PRV at a concentration and for a duration sufficient to cause infection.

Considerations include Sockeye Salmon susceptibility to PRV infection; PRV infection dynamics; oceanographic and environmental conditions; PRV minimum infectious dose; estimated PRV waterborne concentration attributable to Atlantic Salmon farms; hydrodynamic dispersal; and estimated potential duration of exposure.

### Sockeye Salmon susceptibility to PRV infection

Sockeye Salmon susceptibility to PRV infection is demonstrated by the following cohabitation study and detections in Sockeye Salmon sampled in the field.

PRV negative Atlantic and Sockeye salmon sentinels cohabitated with western North American PRV positive Atlantic Salmon donors became infected with the virus after four weeks of cohabitation in seawater (Garver et al., 2016a) providing evidence of Sockeye Salmon susceptibility to PRV. Other studies also reported PRV infections in Sockeye Salmon but through intraperitoneal injections (Garver et al., 2016b; Polinski et al., 2016) which do not mimic natural transmission pathways.

Sockeye Salmon appears to be less susceptible to PRV infections than Atlantic Salmon given lower prevalence and viral load and given that infections appear to take longer to develop (Garver et al., 2016a; Polinski and Garver, *in preparation*). For instance, based on a cohabitation study with PRV-infected Atlantic Salmon, 40% (4/10) of Sockeye Salmon sentinels compared to 100% (15/15) of Atlantic Salmon sentinels became infected with PRV after four weeks of cohabitation (Garver et al., 2016a). Additionally, PRV viral load peaked in 12 weeks in Sockeye Salmon sentinels compared to 6 weeks in Atlantic Salmon sentinels, and maximum viral loads remained lower in blood and kidney in Sockeye Salmon sentinels compared to Atlantic Salmon sentinels (Garver et al., 2016a). Finally, some Sockeye Salmon appeared to be refractory to PRV infection or we able to clear the infection (Garver et al., 2016a).

PRV genetic material has also been detected in Fraser River Sockeye Salmon sampled in BC waters (Jeffries et al., 2014; Miller et al., 2014; Marty et al., 2015; Furey, 2016; Morton et al., 2017; Teffer et al., 2017; Stevenson, 2018).

### Infection dynamics of PRV

Polinski and Garver (*in preparation*) summarized the dynamics of PRV infections as observed in Atlantic Salmon in three main phases: (1) early entry and dissemination; (2) peak systemic replication; and (3) long-term persistence.

- During the early entry and dissemination phase, which typically lasts two to three weeks at 12°C, the virus enters the host, replicates and disseminates into blood cells. The virus

is not likely being shed into the environment to a high degree during this phase (Polinski et al., in press).

- During the peak systemic replication phase, which typically lasts two to three weeks at 12°C, substantial PRV replication takes place within erythrocytes (Finstad et al., 2014; Wessel et al., 2015; Haatveit et al., 2017; Polinski et al., in press) leading to the highest systemic blood loads of PRV.
- During the long-term persistence phase there is a reduction in viral protein production but large quantities of genomic PRV material remain associated with the erythrocyte cell fraction (Haatveit et al., 2017; Lund et al., 2017; Polinski et al., in press). Shedding of the virus is thought to be minimal during this phase and may even cease entirely over time (Garver et al., 2016a).

#### **Oceanographic and environmental conditions**

Water temperatures in the Discovery Islands area vary both seasonally and regionally with recorded temperatures ranging between 3 and 24°C (Chandler et al., 2017). Monthly water temperature in the top 15 m of Atlantic Salmon farms in the Discovery Islands area ranges from 7.6 ± 2.3°C to 11.5 ± 3.3°C (mean ± std) (Chandler et al., 2017).

Water salinity in the Discovery Islands area varies considerably by season (due to river runoff of snowmelt), by depth (due to the estuarine circulation), and by location (as some narrow channels are extremely well mixed vertically) ranging from close to zero to 32. Monthly salinity in the top 15 m of Atlantic Salmon farms in the Discovery Islands area ranges from 28.9 ± 7.3 to 29.9 ± 8.7 (mean ± std) (Chandler et al., 2017).

Whether salinity or temperature influences the survival of PRV in the marine environment is not known. However, the transmission of PRV to Atlantic Salmon in the Discovery Islands area demonstrates that the oceanographic and environmental conditions are conducive for transmission.

#### **PRV minimum infectious dose**

No studies have attempted to determine the minimum dose required to infect Sockeye Salmon with PRV.

In Atlantic Salmon, preliminary evidence using PRV from Pacific Canada suggests that  $\leq 10^3$  PRV particles are sufficient to initiate infection by intra-peritoneal injection (Polinski, unpublished data reported in Polinski and Garver (*in preparation*)). However, injections are not representative of natural exposure and consequently the amount of PRV known to cause infection by injection cannot be extrapolated to a more environmental relevant exposure route.

In Pink Salmon (*O. gorbuscha*), bath exposures to 1,000 purified PRV particles per mL for one hour failed to infect 1 g fish (n=20) in seawater up to six weeks after exposure (Richard, Polinski, and Garver, unpublished data reported in Polinski and Garver (*in preparation*)), providing a better representation of a natural exposure route.

The minimum dose required to induce PRV infection by immersion or ingestion in Sockeye Salmon remains unknown, but is likely dependent upon the route of virus exposure, host condition, stock, and species (Polinski and Garver, *in preparation*).

#### **Estimated PRV waterborne concentration attributable to Atlantic Salmon farms**

Quantifying the infection pressure from an infected farm requires estimations of the number of infected fish on farm, the shedding rate in infected-fish and the volume of the farm.

Although the average volume of Atlantic Salmon farms in the Discovery Islands area has been estimated to be approximately 195,000 m<sup>3</sup> (Mimeault et al., 2017) and that PRV prevalence on an infected Atlantic Salmon farm can be expected to reach 100% at some point within the production cycle (see Exposure Assessment), the viral shedding rate in PRV-infected Atlantic Salmon (or other salmonids) has not been quantified (Polinski and Garver, *in preparation*). Consequently, it is not possible to estimate the infection pressure from a PRV-infected Atlantic Salmon farm in the Discovery Islands area.

#### Hydrodynamic dispersal

Modelling the hydrodynamic dispersion of a pathogen in the marine environment requires an ocean and circulation model, the infection pressure attributable to the source and information about the survival of the pathogen in the marine environment.

There is an existing ocean and circulation model available for the Discovery Islands area (Foreman et al., 2012) that has been used to model hydrodynamic dispersion of infectious hematopoietic necrosis virus (IHNV) between farms (Foreman et al., 2015a) and dispersion of IHNV (Mimeault et al., 2017) and *Aeromonas salmonicida* (Mimeault et al., *in review-a*) in the Discovery Islands area.

Nevertheless, it was not possible to model the dispersal of PRV from infected Atlantic Salmon farms in the Discovery Islands area for this risk assessment given that the viral infection pressure attributable to a PRV-infected farm cannot be estimated (see section on Estimated PRV concentration attributable to Atlantic Salmon farms) and there are no data on the survival (or decay rate) of PRV in the marine environment (Polinski and Garver, *in preparation*).

#### Estimated duration of exposure

The potential duration that Fraser River Sockeye Salmon could be exposed to PRV released from an Atlantic Salmon farm in the Discovery Islands area depends on the time Fraser River Sockeye Salmon spend in the Discovery Islands area in proximity of infected farm(s) and the time an infected farm remains infectious.

##### *Duration of PRV infections on Atlantic Salmon farms*

Once infected, the on-going persistence of PRV infections in Atlantic Salmon has been demonstrated over 59 weeks under experimental conditions (Garver et al., 2016a) and five months under field conditions (Di Cicco et al., 2017). However, as mentioned in the Release Assessment section, it is hypothesized that horizontal transmission primarily occurs between 3 to 15 weeks following infection, after which the potential for natural shedding becomes severely reduced (Polinski and Garver, *in preparation*).

##### *Residence time of Fraser River Sockeye Salmon in Discovery Islands area*

Grant et al. (2018) estimated the residence time of juvenile and adult Sockeye Salmon in the Discovery Islands area, from which Mimeault et al. (2017) estimated, assuming a constant migration speed and unidirectional movement, that juveniles could encounter farms over three to eight days while returning adults could encounter farms over two days during their migration through the Discovery Islands area.

##### *Fraser River Sockeye Salmon in proximity to Atlantic salmon farms*

In a recent telemetry study, the median travel time of juvenile Fraser River Sockeye Salmon (n=21) from Hoskyn Channel to Okisollo Channel (approximately 25 km and including seven salmon farms) was estimated to be 31 hours (Rechisky et al., 2018). From the eastern to the western end of the Okisollo Channel (approximately 4 km and including three salmon farms)



median travel time of juvenile Fraser River Sockeye Salmon (n=20) was estimated to be 6 hours. In the same study, the median time juvenile Sockeye Salmon spent near the Venture Point (n=17) or Brent Islands (n=13) farms were respectively 4.5 minutes (range: 0 to 12) and 4.2 minutes (range: 0 to 24) (Rechisky et al., 2018) suggesting short exposure time. However, given that farms were fallow at the time of the study (Rechisky et al., 2018), it is possible that exposure time would be different when farms are stocked.

## Assumptions

- Sockeye Salmon have been exposed to PRV released from Atlantic Salmon farm(s) in the Discovery Islands area;
- All Fraser River Sockeye Salmon are assumed to be equally susceptible to PRV regardless of life stage or stock of origin;
- Juvenile and adults Fraser River Sockeye Salmon are considered naïve to PRV when migrating through the Discovery Islands area; and
- PRV is dispersed throughout the Discovery Islands area from infected Atlantic Salmon farms.

## Likelihood of infection

Table 12 presents the main factors contributing and limiting the likelihood that Fraser River Sockeye Salmon would become infected with PRV released from Atlantic Salmon farm(s) located in the Discovery Islands area. Those factors were used to determine likelihood and uncertainty rankings based on definitions in Tables 2, 5 and 6.

*Table 12. Factors contributing to and limiting the likelihood that Fraser River Sockeye Salmon would become infected with PRV released from infected Atlantic salmon farms in the Discovery Islands area under current farm practices.*

Contributing factors	Limiting factors
<ul style="list-style-type: none"> <li>• Sockeye Salmon are susceptible to PRV;</li> <li>• Based on juvenile swimming speed and distance it is estimated that juvenile Sockeye Salmon could encounter Atlantic Salmon farms over three to eight days during their migration through the Discovery Islands area;</li> <li>• It is estimated that returning adult Sockeye Salmon could encounter Atlantic Salmon farms over two days during their migration through the Discovery Islands area;</li> <li>• All Atlantic Salmon farms in the Discovery Islands area are anticipated to become infected with PRV within 100-200 days post-seawater transfer; and</li> <li>• PRV prevalence in farmed Atlantic Salmon in the marine environment is</li> </ul>	<ul style="list-style-type: none"> <li>• Based on a telemetry tracking study, juvenile Sockeye Salmon spend limited time (minutes) in the vicinity of fallowed farms;</li> <li>• Median travel time of juvenile Fraser River Sockeye Salmon (n=21) from Hoskyn Channel to Okisollo Channel (approximately 25 km and including seven salmon farms) was estimated to be 31 hours;</li> <li>• Based on laboratory studies, PRV-infected Atlantic Salmon appear to be most contagious between 3 and 15 weeks following PRV infection, after which the potential for horizontal transmission is severely reduced; and</li> <li>• Sockeye Salmon appears to be less susceptible to PRV infections than Atlantic Salmon given lower prevalence and viral load and given that infections appear to take longer to develop.</li> </ul>



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expected to reach 100% approximately 200-300 days post seawater transfer.	
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696 Likelihood of infection was considered for two exposure groups: (1) juvenile Fraser River  
697 Sockeye Salmon; (2) adult Fraser River Sockeye Salmon.

698 It was concluded that the likelihood of at least one Fraser River Sockeye Salmon, at either the  
699 juvenile or adult life stage, to become infected with PRV attributable to Atlantic Salmon farms in  
700 the Discovery Islands area through waterborne exposure under the current farm practices is  
701 **very likely** given that Sockeye Salmon are susceptible to PRV infection and have been shown  
702 to become infected in cohabitation studies. This conclusion was made with **high uncertainty**  
703 given incomplete and highly variable data and that expert opinions vary considerably. Whether  
704 exposure to PRV at environmentally relevant concentrations around the farms and for the period  
705 of time that Fraser River Sockeye Salmon migrate through the Discovery Islands area where  
706 farms are present (three to eight days for juveniles, two days for adults) will result in infection in  
707 Sockeye Salmon is not known.

#### 708 OVERALL LIKELIHOOD ASSESSMENT

709 The estimated likelihoods were combined as per the combination rules described in the  
710 methodology section. The combined likelihood for the release assessment was determined by  
711 adopting the highest likelihood ranking among the release pathways. The combined likelihood  
712 for each exposure group was determined by adopting the lowest ranking among the farm  
713 infection, release, exposure and infection assessments.

714 Table 13 summarizes the likelihood assessment. Overall, it was concluded that the likelihood  
715 that at least one Fraser River Sockeye Salmon would become infected with PRV released from  
716 Atlantic Salmon farms in the Discovery Islands area is **very likely** for both exposure groups.  
717 This conclusion is driven by the likelihood of infection which is highly uncertain given the lack of  
718 data about PRV shedding rates from PRV-infected Atlantic Salmon and the minimum dose of  
719 PRV required to infect Sockeye Salmon.

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*Table 13. Summary of the likelihood and uncertainty rankings for the likelihood assessment of the piscine orthoreovirus risk assessment. Descriptions of the uncertainties can be found with each likelihood assessment steps; uncertainties are not combined. Estimates are reported in white cells and likelihood combination results are reported shadowed cells under the "Rankings" column.*

Steps		Rankings	
<b>Farm infection assessment</b>	<b>Likelihood of farm infection</b>	Extremely likely (high certainty)	
<b>Release assessment</b>	<b>Release pathways</b>	<b>Farmed Atlantic Salmon</b>	<b>Mechanical vectors and fomites</b>
	<b>Likelihood of release</b>	Extremely likely (high certainty)	Unlikely (reasonable uncertainty)
	<b>Combined likelihoods of release</b>	Extremely likely	
<b>Exposure and infection assessments</b>	<b>Exposure groups</b>	<b>At least one juvenile Fraser River Sockeye Salmon</b>	<b>At least one adult Fraser River Sockeye Salmon</b>
	<b>Likelihood of exposure</b>	Extremely likely (reasonable certainty)	Extremely likely (reasonable certainty)
	<b>Likelihood of infection</b>	Very likely (high uncertainty)	
<b>Combined exposure and infection likelihoods for each exposure group</b>		Very likely	Very likely
<b>Combined likelihoods (farm infection, release, exposure and infection) for each exposure group</b>		Very likely	Very likely

## CONSEQUENCE ASSESSMENT

The consequence assessment aims to determine the potential magnitude of impacts of PRV attributable to Atlantic Salmon farms in the Discovery Islands area on the abundance and diversity of the Fraser River Sockeye Salmon.

Based on the likelihood assessment, it was determined that it is very likely that at least one Fraser River Sockeye Salmon would become infected with PRV released from Atlantic Salmon farms in the Discovery Islands area given that all farms could become infected with PRV after seawater transfer of Atlantic Salmon, that PRV infections could happen at any months of the year, and that infections can persist and given Sockeye Salmon susceptibility to PRV infection.

Assuming that at least one Fraser River Sockeye Salmon would have been infected with PRV attributable to infected Atlantic Salmon farms, the consequence assessment explores the potential magnitude of impacts to the number of returning adults and diversity of Fraser River Sockeye Salmon.

**QUESTION**

Assuming that at least one susceptible Fraser River Sockeye Salmon has been infected with PRV released from infected Atlantic Salmon, what is the potential magnitude of impact on the number of returning adults and diversity of Fraser River Sockeye Salmon?

**CONSIDERATIONS**

Considerations include pathogenicity and virulence of PRV; and PRV prevalence in Sockeye Salmon.

**Pathogenicity and virulence of PRV**

To date, PRV1 is the only genogroup detected in North America (Polinski and Garver, *in preparation*) hence the focus of this risk assessment. Refer to Polinski and Garver (*in preparation*) for a summary of the state of knowledge related to the pathogenicity of other PRV genogroups in different salmonid species and regions.

Briefly, PRV1 has been demonstrated to be an etiological component of heart and skeletal muscle inflammation (HSMI) in farmed Atlantic Salmon in Norway (Wessel et al., 2017) and is a putative contributing factor in severe cardiomyopathy in farmed Atlantic Salmon in Pacific Canada (Di Cicco et al., 2017; Di Cicco et al., 2018). PRV1 has also been suggested to be a contributing factor in jaundice/anemia in farmed Chinook Salmon (*O. tshawytscha*) in Pacific Canada (Di Cicco et al., 2018).

However, under experimental conditions with Atlantic Salmon, PRV from Pacific Canada was highly infectious but did not cause HSMI (Garver et al., 2016a), did not result in impaired respiratory function (Zhang et al., *in press*) and was of low virulence causing only minor focal heart inflammation without significant transcriptional induction of immune genes (Polinski et al., *in press*).

**Farmed Atlantic Salmon**

In Norway, most farmed Atlantic Salmon become PRV positive but only some develop the disease (Polinski and Garver, *in preparation*). While HSMI is common in farmed Atlantic Salmon in Norway (Kongtorp et al., 2004a; Kongtorp et al., 2004b; Kongtorp et al., 2006; Palacios et al., 2010), it is not clear why some experience high losses and others do not (Polinski and Garver, *in preparation*).

In contrast, while most farmed Atlantic Salmon in Pacific Canada also become PRV positive, clinical HSMI outbreaks as in Norway have not been reported (Polinski and Garver, *in preparation*) but subclinical farm-level cases of HSMI-like disease have been suggested for which PRV may or may not be a causative factor (Di Cicco et al., 2017; Di Cicco et al., 2018; Polinski et al., *in press*).

No fish health events nor mortality events have been attributed to HSMI on Atlantic Salmon farms in BC.

**Sockeye Salmon**

Of most relevance to this risk assessment are the consequences of PRV infection in Sockeye Salmon. To date, there is no evidence that PRV causes disease in Sockeye Salmon despite successful infection with the virus under experimental conditions (Garver et al., 2016a; Garver et al., 2016b; Polinski et al., 2016).

Sockeye Salmon post-smolts (40 g) challenged by intraperitoneal injections with a PRV inoculum prepared from infectious Atlantic Salmon developed considerable blood and kidney PRV loads but no weight loss, morbidity or pathology could be attributed to the virus over 62 days after challenge (Polinski et al., 2016). Despite high viral loads, PRV only induced a weak host response in head kidneys within the first three to four weeks of infection and the presence of PRV did not change the host response to a superinfection with infectious hematopoietic necrosis virus (Polinski et al., 2016).

In another laboratory study, PRV negative Atlantic and Sockeye salmon (sentinels) were cohabitated in seawater with PRV positive Atlantic Salmon (75 g) (donors) injected with an inoculum prepared from highly PRV infective Atlantic Salmon. Despite high prevalence and persistence of PRV in blood and kidney of both sentinel species over 59 weeks, no microscopic lesions, disease or mortality could be attributed to the virus (Garver et al., 2016a).

Chinook Salmon, Sockeye Salmon and Atlantic Salmon challenged by intraperitoneal injections with a PRV inoculum prepared from jaundiced Chinook Salmon did not develop clinical jaundice despite testing positive for PRV five months after challenge (Garver et al., 2016b).

Finally, preliminary data indicate that PRV infections are inconsequential to Sockeye Salmon respiratory function (Polinski et al. in preparation reported in Polinski and Garver (*in preparation*)).

Overall, the results from the above laboratory studies suggest that PRV from Pacific Canada is infectious but of low virulence to Sockeye Salmon (Garver et al., 2016a; Polinski et al., 2016; Polinski and Garver, *in preparation*). Additionally, the presence of PRV on or in the gills had no significant effects on the likelihood that returning adult Fraser River Sockeye Salmon from Chilko or Shuswap Lake stocks would reach their spawning grounds (Miller et al., 2014).

## **PRV prevalence in Sockeye Salmon**

Polinski and Garver (*in preparation*) summarized PRV screening results in Pacific Salmon sampled from Alaska, British Columbia and Washington from which they estimated an overall PRV prevalence of 1.4% in Sockeye Salmon based on results from 12 independent studies.

Table 14 summarises the PRV screening and positive detection in Sockeye Salmon per life stage and environment. Of the 6693 Sockeye Salmon screened for PRV, 4725 have been attributed to the Fraser River. With a total of 86 positive detections in Fraser River Sockeye Salmon, the overall PRV prevalence in Fraser River Sockeye Salmon is estimated to be 1.8%. Most positive detections were reported in returning adults (83/86) with respective PRV prevalence of 0.1% and 4.2% in juveniles and adults Fraser River Sockeye Salmon.

PRV prevalence in juvenile Fraser River Sockeye Salmon is similar in freshwater (0.1%) and seawater (0.2%) while in returning adults PRV prevalence in freshwater (1.3%) is lower than in seawater (12.1%). However, 98% (63/64) of positive detections in returning adult Fraser River Sockeye Salmon sampled in seawater were from gill biopsies and might not all be indicative of systemic infections as liver samples taken at the time of gill biopsies, as well as subsequently in the Fraser River, were negative (Polinski and Garver, *in preparation*).

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Table 14. PRV screening and positive detections in Sockeye Salmon of Alaska, British Columbia (BC), and Washington by life stage and/or sampling environment. Adapted from Polinski and Garver (in preparation) which includes results from Jeffries et al. (2014); Miller et al. (2014); Marty et al. (2015); Furey (2016); Morton et al. (2017); Teffer et al. (2017); Nekouei et al. (2018); Purcell et al. (2018); Stevenson (2018); Thakur et al. (in press); Hrushow (2018); and Johnson (unpublished).

Sockeye Salmon		Number of PRV positive fish/number fish screened (%)					Total
		Fry	Juveniles		Adults		
		Freshwater	Freshwater	Seawater	Seawater	Freshwater	
Alaska, BC and Washington	By group	3/89 (3.4%)	1/1879 (0.1%)	8/1943 (0.4%)	64/560 (11.4%)	21/2352 (0.9%)	97/6693 (1.4%)
	Sub-total	3/89 (3.4%)	9/3822 (0.2%)		85/2912 (2.9%)		
Fraser River Sockeye Salmon only	By group	--	1/1505 (0.1%)	2/1258 (0.2%)	64/531 (12.1%)	19/1431 (1.3%)	86/4725 (1.8%)
	Sub-total		3/2763 (0.1%)		83/1962 (4.2%)		

Polinski and Garver (in preparation) also summarized PRV screening results by Fraser River Sockeye Salmon stocks. To date, of the 4725 Fraser River Sockeye Salmon screened for PRV, 4337 have been genetically attributed to specific stocks from the Fraser River, representing 22 of the 24 Fraser River Sockeye Salmon conservation units (CUs) (Table 15).

Positive detections have been reported in six Fraser River Sockeye Salmon stocks (Adams, Chilko Lake, Cultus Lake, Nadina River, Stuart Lake and Shuswap Lake) representing five to seven of the 24 conservation units. Given the low sample size of fish screened for PRV in some stocks and absence of screening for PRV in other stocks, PRV may also be present in other stocks and conservation units.

Table 15. Distribution of PRV detection across Fraser River Sockeye Salmon stocks and the 24 Wild Salmon Policy Conservation Units. Sources: 2017 integrated biological status as per DFO (2018a). PRV screening results as per Jeffries et al. (2014); Miller et al. (2014); Marty et al. (2015); Furey (2016); Morton et al. (2017); Teffer et al. (2017); Nekouei et al. (2018); Stevenson (2018). EStu: Early Stuart; ES: Early Summer; S: Summer; L: Late; NA: Not applicable, --: no tests, \* questionable positive detection in Marty et al. (2015).

2017 status		Conservation unit-Management unit	Stock screened for PRV	PRV screening results	
				Juveniles	Adults
Red		Bowron-ES	Bowron	0/9	--
Red		Cultus-L	Cultus	1/62	--
Red		Taseko-ES	--	--	--
Red		Widgeon-River	--	--	--
Red		Harrison (U/S)-L	Weaver	0/8	--
Red		Seton-L	Portage	0/35	--
Red		Takla-Trembleur-EStu	Early Stuart, Late Stuart & Misc. <sup>1</sup>	0/4	1/191
R	A	Takla-Trembleur-Stuart-S			
R	A	Quesnel-S	Quesnel	0/22	0/297
			Horsefly	0/148	--
			Mitchell	0/119	--
			Blue Lead	0/1	--
			Wasko-Roaring	0/16	--

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Amber		Nahatlatch-ES	Nahatlatch River	0/16	--
Amber		North Barriere-ES	Fennell	0/1	--
Amber		Kamloops-ES	Thompson	0/75	--
			Raft	0/18	--
			Upper Barrier	0/3	--
Amber		Lillooet-Harrison-L	Birkenhead	0/77	0/11
Amber		Shuswap-ES	Scotch <sup>2</sup>	0/72	0/8
			Seymour <sup>2</sup>	0/134	--
A	G	Shuswap Complex-L	Adams	1/370	0/2
			Shuswap <sup>3</sup>	0/398	49/304
			Eagle	0/6	--
			Little	0/5	--
A	G	Nadina-Francois-ES	Nadina	0/60	1 <sup>4</sup> /60
A	G	Chilliwack-ES	Dolly Varden	0/86	--
			Chilliwack Lake	0/34	--
A	G	Francois-Fraser-S	Stellako	0/137	0/10
A	G	Anderson-Seton-ES	Gates	0/65	0/19
A	G	Harrison (D/S)-L	Big Silver	0/4	--
Green		Pitt-ES	Pitt	0/79	--
Green		Harrison River - River	Harrison <sup>5</sup>	--	0/103
Green		Chilko-S and Chilko-ES	Chilko <sup>6</sup>	0/1018	15/250
DD		Chilko-ES			
Sub-total by life stage				2/3082 (0.1%)	66/1255 (5.3%)
Total				68/4337 (1.6%)	

837 <sup>1</sup> We are unable to distinguish whether samples identified as belonging to the Stuart stock are part of the  
838 "Takla-Trembleur-ES" CU or the "Takla-Trembleur-Stuart-S" CU. We also include juvenile fish sampled  
839 from Sandpoint Creek, Five Mile Creek, Middle River, and Dist-Sinta Creek (n=1 per stock) as part of this  
840 combined TTE or TTS CU.

841 <sup>2</sup> We have assumed that samples identified as belonging to Scotch Creek and Seymour River are from  
842 the early summer timed CU "Shuswap-ES"; however we note that both of these streams also produce a  
843 smaller late-timed run that is part of the "Shuswap Complex-L" CU.

844 <sup>3</sup> We have included stocks from the Middle Shuswap River (n=53) in this categorization, although it is  
845 possible that some of these fish may be of the Shuswap-ES CU.

846 <sup>4</sup> Positive detection of PRV nucleic acid in only one of two technical replicates which was noted as  
847 inconclusive by the authors (Mary et al., 2015).

848 <sup>5</sup> We have assumed that adult samples identified as belonging to the Harrison stock are part of the  
849 "Harrison River - River" CU.

850 <sup>6</sup> We are unable to distinguish whether samples identified as belonging to the Chilko stock are part of the  
851 "Chilko-S" CU or the "Chilko-ES" CU.

## 852 ASSUMPTIONS

- 853 • Results from laboratory studies on the impact of PRV infection in Sockeye Salmon are  
854 indicative of what occurs in the marine environment;

- 855 • Prevalence of PRV in sample is representative of the prevalence of the whole stock in all  
856 years;
- 857 • Juvenile and adult Fraser River Sockeye Salmon are assumed to be equally susceptible to  
858 PRV; and
- 859 • All Fraser River Sockeye Salmon stock have the same susceptibility.

## 860 **MAGNITUDE OF CONSEQUENCES**

861 The consequence assessment explores the potential magnitude of impact to the abundance  
862 and diversity of Fraser River Sockeye Salmon resulting from juvenile and adult Fraser River  
863 Sockeye Salmon infected with PRV released from Atlantic Salmon from all farms located in the  
864 Discovery Islands area.

865 The likelihood assessment concluded that it is very likely that at least one Fraser River Sockeye  
866 Salmon would get infected with PRV attributable to Atlantic Salmon farms in the Discovery  
867 Islands area. It is however not possible to determine the proportion of migrating Fraser River  
868 Sockeye Salmon that would get infected with PRV given the significant knowledge gaps related  
869 to the estimation of the PRV infection pressure from an Atlantic Salmon farm, the minimum PRV  
870 dose required to infect Sockeye Salmon and the interactions of Sockeye Salmon with Atlantic  
871 Salmon farms. Predicated on the prevalence of PRV in the population, any effects, if any, would  
872 be limited to the fish infected with PRV attributable to Atlantic Salmon farms in the Discovery  
873 Islands area.

874 The potential magnitude of consequences on both the abundance and diversity of Fraser River  
875 Sockeye Salmon resulting from infection with PRV attributable to Atlantic Salmon farms in the  
876 Discovery Islands area was determined for juvenile and adult Fraser River Sockeye Salmon.  
877 Rankings were determined referring to definitions of consequence to abundance (Table 3),  
878 consequences to diversity (Table 4) and uncertainty (Table 5).

## 879 **Juvenile Fraser River Sockeye Salmon**

880 Lake-type juvenile Fraser River Sockeye Salmon migrate through the Discovery Islands area  
881 during their outmigration. Given the ubiquitous nature of PRV on Atlantic Salmon farms and its  
882 high prevalence and persistence on infected farms, it was concluded that it is very likely that at  
883 least one juvenile Fraser River Sockeye Salmon would become infected during their  
884 outmigration. However, it is not possible to determine the proportion of the juveniles that could  
885 become infected nor the potential for an infection acquired in the Discovery Islands area to  
886 spread to other juvenile Fraser River Sockeye Salmon during migration at sea.

887 However, although the proportion of juvenile Fraser River Sockeye Salmon getting infected with  
888 PRV attributable to Atlantic Salmon farms is unknown, only two positive PRV detections have  
889 been reported in juvenile Fraser River Sockeye Salmon sampled in seawater over a total of  
890 1258 (0.2%) (Polinski and Garver, *in preparation*) (Table 14). Whether this low prevalence in  
891 juveniles sampled at sea is an artefact of the short time period between potential infection and  
892 screening is unknown but is a possibility as Garver et al. (2016a) demonstrated that several  
893 weeks post exposure are necessary for detection of the virus in both Atlantic and Sockeye  
894 salmon under experimental conditions. Regardless of the proportion infected, PRV from Pacific  
895 Canada appears to be infectious but of low virulence under laboratory conditions (Garver et al.,  
896 2016a; Garver et al., 2016b; Polinski et al., 2016; Polinski and Garver, *in preparation*).

897 Overall, the low prevalence, low virulence, absence of impact on respiratory performance of  
898 PRV in Sockeye Salmon suggest a limited impact of PRV on the survival of Fraser River  
899 Sockeye Salmon. It was therefore concluded that the potential magnitude of consequences to



the abundance of Fraser River Sockeye Salmon would be **negligible**. This conclusion was made with **reasonable certainty** given abundant and robust data on the low prevalence and virulence of PRV in Sockeye Salmon.

Juvenile Fraser River Sockeye Salmon from 22 conservation units have been screened for PRV. Two PRV positive detections have been reported: one in the Cultus-L and one in the Shuswap Complex-L conservation units (Table 14). However, given the low prevalence and virulence of the virus in juvenile Sockeye Salmon, it was concluded that the potential magnitude of consequences to the diversity of Fraser River Sockeye Salmon would be **negligible** over two generations (eight years). This conclusion was made with **reasonable certainty** given abundant and robust data on the low prevalence and virulence of PRV in Sockeye Salmon.

### Adult Fraser River Sockeye Salmon

In any given year, between 10 and 96% of returning adult Fraser River Sockeye Salmon migrate through the northern diversion route (Grant et al., 2018) and hence could be exposed to an Atlantic Salmon farm in the Discovery Islands area. Given the ubiquitous nature of PRV on Atlantic Salmon farms and its high prevalence and persistence on infected farms, it was concluded that it is very likely that at least one adult Fraser River Sockeye Salmon would become infected during their outmigration through the Discovery Islands area, it is however not possible to determine the proportion of adults that could become infected due to Atlantic Salmon farms in the Discovery Islands area.

Overall, the average PRV prevalence in adult Fraser River Sockeye Salmon is 4.2% with a maximum of 12.1% in seawater. However, most (63/64) of the PRV positive detections reported in returning adult Fraser River Sockeye Salmon sampled in seawater were from gill biopsies (Miller et al., 2014). Liver samples taken at the same time of gill biopsies as well as subsequently in the Fraser River were negative for PRV; suggesting that the PRV on or in the gill tissues of these fish did not represent systemic infections nor did systemic infections likely develop before returning fish reached their spawning grounds (Polinski and Garver, *in preparation*).

Returning Fraser River Sockeye Salmon can travel the distance between the southeastern limit of the Discovery Islands area and Mission in approximately three to four days (Grant et al., 2018). The distance between Fraser River Sockeye Salmon spawning grounds and the ocean ranges widely, from 40 km for the Widgeon Slough population to 1,200 km for the Early Stuart population (Cohen, 2012b). Early Stuart River Sockeye Salmon took up to a month to reach their spawning grounds from the mouth of the Fraser River (Stoddard, 1993). Consequently, depending on the stocks, returning adults can take up to 35 days to reach their spawning grounds.

Given that under experimental conditions Sockeye Salmon required four weeks to develop detectable PRV infections through cohabitation with Atlantic Salmon donors (Garver et al., 2016a), that PRV transmission likely takes more than three weeks to occur following infection (Polinski and Garver, *in preparation*) and that PRV prevalence in adult Fraser River Sockeye Salmon sampled in freshwater is 1.3%, no significant spread of infection within the returning adults prior to spawning is expected.

PRV infections had no significant effects on the likelihood that returning adult Fraser River Sockeye Salmon from two different stocks would reach their spawning grounds (Miller et al., 2014). In absence of additional data specific to PRV infections in adult Sockeye Salmon, surrogate data based on different species or different life stages were also considered:



- Notwithstanding that PRV responses vary between salmon species, there are only rare occurrences of diseases associated with PRV in farmed Atlantic Salmon in BC despite the ubiquitous nature and high prevalence of the virus; and
- Based on laboratory studies conducted with juvenile Sockeye Salmon, PRV from Pacific Canada appears to be infectious but of low virulence under laboratory conditions (Garver et al., 2016a; Garver et al., 2016b; Polinski et al., 2016; Polinski and Garver, *in preparation*), hence PRV is also expected to be of low virulence in adults.

Overall, regardless of the proportion of returning adult Fraser River Sockeye Salmon infected with PRV, the low prevalence, low virulence and absence of significant impact on the likelihood of reaching spawning grounds in PRV-infected Sockeye Salmon suggest a limited impact of PRV on the survival of Fraser River Sockeye Salmon. It was therefore concluded that the potential magnitude of consequences to the abundance of Fraser River Sockeye Salmon would be **negligible**. This conclusion was made with **reasonable uncertainty** given abundant and robust data on the low virulence of PRV in Sockeye Salmon but reliance on surrogate data for determining potential consequences.

Adult Fraser River Sockeye Salmon from nine conservation units have been screened for PRV. Positive detections were reported in four stocks representing four to six of the 24 Fraser River Sockeye Salmon conservation units (Table 14). However, since no significant spread of infection within the returning adults prior to spawning is expected and given the low virulence of the virus in Sockeye Salmon, it was concluded that the potential magnitude of consequences to the diversity of Fraser River Sockeye Salmon would be **negligible** over two generations (eight years). This conclusion was made with **reasonable uncertainty** given abundant and robust data on the low virulence of PRV in Sockeye Salmon but reliance on surrogate data for determining potential consequences.

## RISK ESTIMATION

### ABUNDANCE

The risk to the abundance of Fraser River Sockeye Salmon due to infections with PRV attributable to Atlantic Salmon farms in the Discovery Islands area (Table 16) was estimated using the risk matrix combining the results of the likelihood assessment and the results of the consequence assessment to Fraser River Sockeye Salmon abundance (Figure 3).

*Table 16. Risk estimation to the abundance of Fraser River Sockeye Salmon resulting from piscine orthoreovirus attributable to Atlantic Salmon farms located in the Discovery Islands area of under current farm practices.*

Exposure group	Likelihood assessment	Consequence assessment	Risk to Fraser River Sockeye Salmon abundance
Juvenile Fraser River Sockeye Salmon	Very likely	Negligible	Minimal
Adult Fraser River Sockeye Salmon	Very likely	Negligible	Minimal

Overall, it was concluded that, under the current farm practices, the risk to the abundance of Fraser River Sockeye Salmon as a result of a PRV infection attributable to Atlantic Salmon farms in the Discovery Islands area is **minimal**.

## DIVERSITY

The risk to the diversity of Fraser River Sockeye Salmon due to infections with PRV attributable to Atlantic Salmon farms in the Discovery Islands area (Table 17) was estimated using the risk matrix combining the results of the likelihood assessment and the results of the consequence assessment to Fraser River Sockeye Salmon diversity (Figure 4).

*Table 17. Risk estimation to the diversity of Fraser River Sockeye Salmon resulting from piscine orthoreovirus attributable to Atlantic Salmon farms located in the Discovery Islands area of under current farm practices.*

Exposure group	Likelihood assessment	Consequence assessment	Risk to Fraser River Sockeye Salmon diversity
Juvenile Fraser River Sockeye Salmon	Very likely	Negligible	Minimal
Adult Fraser River Sockeye Salmon	Very likely	Negligible	Minimal

It was concluded that, under the current farm practices, the risk to the diversity of Fraser River Sockeye Salmon as a result of a PRV infection attributable to Atlantic Salmon farms in the Discovery Islands area is **minimal**.

## SOURCES OF UNCERTAINTIES

Total uncertainty includes both variability, which is a function of the system that is not reducible with additional measurements, and lack of knowledge that may be reduced with additional data or expert opinion (Vose, 2008). There are uncertainties associated with both the likelihood and consequence assessments.

## LIKELIHOOD ASSESSMENT

The main uncertainties related to the likelihood assessment are attributed to:

- the source(s) and survival of PRV in the marine environment is unknown;
- the variability and knowledge gaps about precise migration routes of lake-type Fraser River Sockeye Salmon through the Discovery Islands area;
- the shedding rates from PRV infected Atlantic Salmon are unknown; and
- the minimal infectious doses of PRV in Sockeye Salmon are unknown.

## CONSEQUENCE ASSESSMENT

The main uncertainties in the consequence assessments for both abundance and diversity resulted from:

- the persistence of PRV infection in Sockeye Salmon is unknown;
- the lack of understanding of how PRV spreads within migrating fish populations; and
- minimal information on PRV prevalence and impact on different conservation units of Fraser River Sockeye Salmon.

## CONCLUSIONS

The assessment concluded that PRV attributable to Atlantic Salmon farms in the Discovery Islands area poses minimal risk to Fraser River Sockeye Salmon abundance and diversity under the current farm practices.

The attribution of the minimal risk was mainly influenced by the potential magnitude of consequences to Fraser River Sockeye Salmon. Despite concluding that it is very likely that Fraser River Sockeye Salmon would become infected with PRV attributable to Atlantic Salmon farms in the Discovery Islands area, the consequence of such infections to both Fraser River Sockeye Salmon abundance and diversity would be expected to be negligible.

There are important sources of uncertainties associated to the determination of the risk to Fraser River Sockeye Salmon due to PRV attributable to Atlantic Salmon farms in the Discovery Islands area. The main uncertainties are related to shedding rate in PRV-infected Atlantic Salmon, PRV survival in the marine environment, and the minimum infectious doses of PRV required to infect Sockeye Salmon. Additionally, there is a lack of knowledge about the persistence of PRV infections in Sockeye Salmon, the spread of infections in migrating Fraser River Sockeye Salmon and impact on different conservation units of Fraser River Sockeye Salmon. Conclusions of this risk assessment should be reviewed as new research findings fill knowledge gaps.

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- 1255



1256

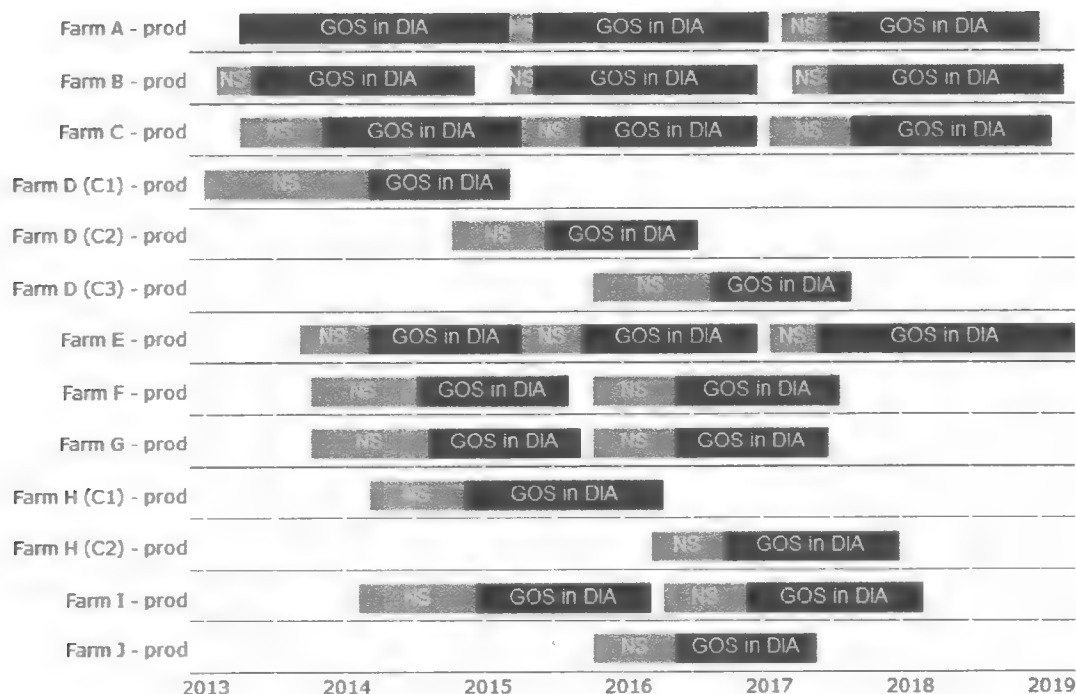
## APPENDICES

### 1257 APPENDIX A: ATLANTIC SALMON PRODUCTION CYCLES IN THE DISCOVERY 1258 ISLANDS AREA

1259 Atlantic Salmon production cycles in the Discovery Islands area were summarized in November  
1260 2018 based on dates of fish transfers between January 2013 and November 2018.

1261 Grow-out periods in the Discovery Islands area ranged between 12 and 23 months (average=17  
1262 months, n=27 cycles) from the beginning of fish transfer to grow-out sites to the end of  
1263 harvesting periods. Fish can be stocked between 2 and 14 months (average=7 months, n=23  
1264 cycles) on nursery sites prior to being transferred to grow-out sites in the Discovery Islands  
1265 area.

1266 Between January 2013 and November 2018, fish transfers to grow-out sites in the Discovery  
1267 Islands area occurred in every month of the year with most of them occurred in May and June  
1268 (Figure 6). Within a given production cycle, fish are usually transferred within a given month but  
1269 can sometime extend over four months.



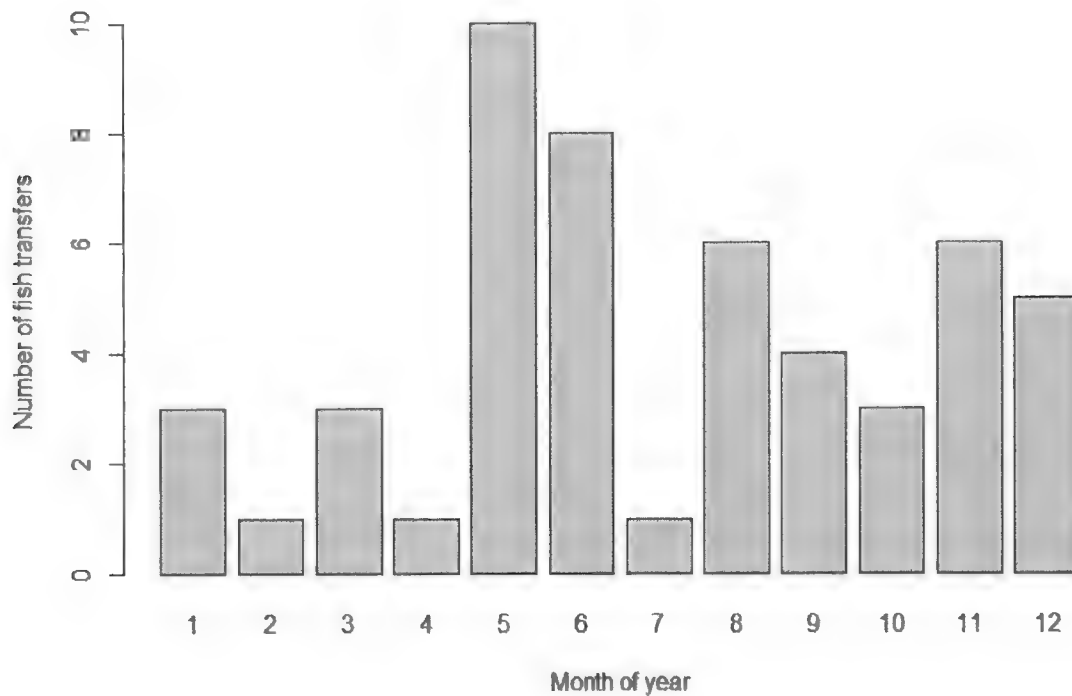
1270

1271 *Figure 6. Production cycles initiate between January 2013 and December 2017 on Atlantic Salmon farms*  
1272 *in the Discovery Islands area. Only marine grow-out sites stocked with fish transferred from seawater*  
1273 *nursery sites are included. For clarity, production cycles on any given farm are represented on a single*  
1274 *row (Farm A to J) or multiple rows when nursery and grow-out periods overlap (farms D and H). Periods*  
1275 *with fish stocked at seawater nursery sites are labelled as "NS" (light blue) and periods of grow-out sites*  
1276 *in the Discovery Islands area are labelled "GOS" (dark blue). Data summarized in November 2018*  
1277 *including predicted harvest dates out to mid-2019. Data source for production cycles: Aquaculture*  
1278 *Management, DFO.*

PRV risk assessment

**DRAFT (DO NOT CITE OR DISTRIBUTE)**

1279



1280

1281 *Figure 7. Atlantic Salmon transfers to marine grow-out sites in the Discovery Islands area between*  
1282 *January 2013 and June 2018. Data includes first transfers over a total of 28 production cycles from*  
1283 *hatcheries and seawater nursery sites to marine grow-out sites. Data provided by Aquaculture*  
1284 *Management, DFO.*

PRV risk assessment

**DRAFT (DO NOT CITE OR DISTRIBUTE)**

1285 **APPENDIX B: DFO AUDIT DEFICIENCIES**

1286 *Table 18. Number of deficiencies identified during audits conducted by Fisheries and Oceans Canada on*  
 1287 *Atlantic Salmon farms 2011-2017 in British Columbia. Data provided by DFO Aquaculture Management*  
 1288 *(updated from Wade, 2017).*

DFO audit deficiency categories	2011	2012	2013	2014	2015	2016	2017	Total
Carcass retrieval protocol or record keeping needs improvement	2	8	4	23	23	21	18	99
Current finfish licence was not posted at facility	0	0	2	0	1	1	3	7
Disease contingency or mass mortality information or records needs improvement	2	1	0	0	0	9	11	23
Fish euthanasia and/or methods not recorded	3	1	0	0	0	0	1	5
Footbaths or sanitizers needs improvement	0	1	3	11	3	4	1	23
Husbandry or record keeping as per COL Appendix VIII-A or VIII-B needs improvement	2	5	4	3	6	2	3	25
Lice protocol or lice records as per COL VII or VII-A needs improvement	21	17	15	18	19	9	26	125
Mooring signage needs improvement	21	6	7	6	9	6	3	58
Mortality assessment or classification needs improvement	0	0	0	0	0	0	0	0
Nutritional or medicated feed protocol concerns	0	0	2	1	3	0	1	7
Training documentation is not up-to-date	0	4	0	3	5	0	1	13
Transfer records are not complete or up-to-date	25	9	9	3	3	3	6	58
Visitor protocol communication needs improvement	7	2	4	2	0	0	1	16
Water quality monitoring, equipment or record keeping needs improvement	0	0	1	0	0	0	1	2
Wild fish mortality records need clarification	0	1	1	0	0	2	3	7
<b>Total # deficiencies</b>	<b>83</b>	<b>55</b>	<b>52</b>	<b>70</b>	<b>72</b>	<b>57</b>	<b>79</b>	<b>468</b>
<b># audits</b>	<b>58</b>	<b>102</b>	<b>96</b>	<b>99</b>	<b>110</b>	<b>106</b>	<b>111</b>	<b>682</b>
<b># farms with deficiencies</b>	<b>40</b>	<b>35</b>	<b>31</b>	<b>41</b>	<b>45</b>	<b>41</b>	<b>29</b>	<b>262</b>
<b>Average # deficiencies/audit</b>	<b>1.43</b>	<b>0.54</b>	<b>0.54</b>	<b>0.71</b>	<b>0.65</b>	<b>0.54</b>	<b>0.71</b>	<b>0.73</b>

1289

## National CSAS Process

### Assessment of the risk to Fraser River sockeye salmon due to piscine orthoreovirus (PRV) transfer from Atlantic salmon farms located in the Discovery Islands area, British Columbia

#### AGENDA

January 28<sup>th</sup> to 30<sup>th</sup>, 2019  
Mount Pleasant Conference Room  
Delta Hotels Vancouver Downtown Suites  
550 West Hastings Street  
Vancouver, British Columbia V6B 1L6

<b>DAY 1</b>		
<b>Monday, January 28, 2019</b>		
13:00 - 13:15	Welcome, Introductions, Housekeeping & Review of Agenda	Gilles Olivier & Craig Stephen (Chairs)
13:15-13:30	CSAS Overview & Meeting Procedures	Gilles Olivier
13:30-13:45	Review Terms of Reference	Craig Stephen
13:45-14:00	Risk assessment process and linkages with the working papers	Ingrid Burgetz
14:00-14:30	<b>Presentation #1:</b> Characterization of piscine orthoreovirus (PRV) and associated diseases to inform pathogen transfer risk assessments in British Columbia	Mark Polinski
<b>BREAK</b>		
14:45-15:45	Reviewer Presentations & Authors Response	Espen Rimstad, Niccolo Vendramin, <i>Ted Meyers</i>
15:45-16:45	Open Discussion & Preparation of Summary Bullets	Everyone
16:45-17:00	Summary & Adjournment	Gilles Olivier
<b>DAY 2</b>		
<b>Tuesday, January 29, 2019</b>		
8:30-8:45	Review of Day 1	Craig Stephen
8:45-9:30	<b>Presentation #2:</b> Assessment of the risk to Fraser River Sockeye Salmon due to piscine orthoreovirus (PRV) on Atlantic Salmon farms in the Discovery Islands area, British Columbia	Caroline Mimeault
9:30-10:30	Reviewer Presentations & Author Response	Ian Gardner, Mark Powell, <i>Edmund Peeler</i>
<b>BREAK</b>		
10:45-12:00	Open Discussion & Preparation of Summary Bullets	Everyone
<b>LUNCH</b>		
13:00-14:00	Open Discussion & Preparation of Summary Bullets	Everyone
14:00-15:00	Science Advisory Report Development	Everyone
<b>BREAK</b>		
15:15-16:30	Science Advisory Report Development	Everyone
16:30-17:00	Summary & Adjournment	Gilles Olivier

Italicized names represent reviewers that provided written comments only

<b>DAY 3</b>		
<b>Wednesday, January 30, 2019</b>		
8:30-8:45	Review of Day 2	Craig Stephen
8:45-10:00	Science Advisory Report Development	Everyone
<b>BREAK</b>		
10:15-12:00	Science Advisory Report Development	Everyone
<b>LUNCH</b>		
13:00-14:30	Science Advisory Report Development	Everyone
14:30-15:15	Final Consensus	Craig Stephen & Gilles Olivier
<b>BREAK</b>		
15:30-17:00	Conclusions & Next Steps	Gilles Olivier

Italicized names represent reviewers that provided written comments only

# **List of Participants**

(Attendees)

Name	Affiliation
	BC Centre for Aquatic Health Sciences
Alistair Struthers	Fisheries and Oceans Canada
	Cermaq Canada
	Pacific Salmon Foundation
Caroline Mimeault	Fisheries and Oceans Canada
	First Nations Fisheries Council of BC
Craig Stephen	Canadian Wildlife Health Cooperative
	Kintama Research
Espen Rimstad	Norwegian University of Life Sciences (NMBU)
France Boily	Fisheries and Oceans Canada
Gary Marty	BC Animal Health Centre
Gilles Olivier	Fisheries and Oceans Canada
	Fish Vet Group
Ian Gardner	Atlantic Veterinary College UPEI
Ingrid Burgetz	Fisheries and Oceans Canada
Jay Parsons	Fisheries and Oceans Canada
	David Suzuki Foundation
Kendra Holt	Fisheries and Oceans Canada
Kristi Miller-Saunders	Fisheries and Oceans Canada
Kyle Garver	Fisheries and Oceans Canada
Lily Weber	Fisheries and Oceans Canada
Mark Polinski	Fisheries and Oceans Canada
Mark Powell	Institute of Marine Research Norway
Myron Roth	BC Ministry of Agriculture
Nathalie Bruneau	Canadian Food Inspection Agency
Nellie Gagne	Fisheries and Oceans Canada
Niccolo Vendramin	Technical University of Denmark
Simon Jones	Fisheries and Oceans Canada
	Veterinary Consultant
Stewart Johnson	Fisheries and Oceans Canada
	Grieg Seafood
Tony Farrell	University of British Columbia
Zac Waddington	Fisheries and Oceans Canada

(Comments only)

Name	Affiliation
Edmund Peeler	Center for Environment Fisheries and Aquaculture Science (CEFAS)
Ted Meyers	Alaska Department of Fish and Game

## Miller-Saunders, Kristi

---

**From:** Malcolm, Gabrielle  
**Sent:** January-16-19 11:37 AM  
**To:** [REDACTED] Struthers, Alistair; Waddington, Zac; Miller-Saunders, Kristi; Gagne, Nellie; 'Bruneau, Nathalie (CFIA/ACIA) (nathalie.bruneau@canada.ca)'; 'myron.roth@gov.bc.ca'; [REDACTED] Olivier, Gilles; 'Craig Stephen'  
**Cc:** Parsons, Jay; Burgetz, Ingrid; Kristmanson, James  
**Subject:** DFO PRV Steering Committee Meeting Minutes  
**Attachments:** SC Meeting Minutes - January 15, 2019 .docx

Dear Steering Committee members,

Please find attached the Minutes from yesterday's call.

If you have any questions, concerns or comments about the Minutes, please do not hesitate to contact me.

Kind regards,

**Gabrielle Malcolm**

Science Advisor, Aquaculture Regulatory Sciences, Aquaculture, Biotechnology and Aquatic Animal Health Science Branch

Fisheries and Oceans Canada / Government of Canada

[gabrielle.malcolm@dfo-mpo.gc.ca](mailto:gabrielle.malcolm@dfo-mpo.gc.ca) / T: 613-949-7466

Conseillère des sciences, sciences de réglementation de l'aquaculture, Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques

Pêches et Océans Canada / Gouvernement du Canada

[gabrielle.malcolm@dfo-mpo.gc.ca](mailto:gabrielle.malcolm@dfo-mpo.gc.ca) / T: 613-949-7466



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## **Piscine orthoreovirus (PRV) CSAS Steering Committee Meeting**

### **Minutes**

Tuesday, January 15<sup>th</sup>, 2019, 1:00-2:00PM (EST)

**Attendees:** Gilles Olivier (Co-Chair), Craig Stephens (Co-Chair), Ingrid Burgetz, Zac Waddington, Alistair Struthers, Nellie Gagne, Nathalie Bruneau, Myron Roth, Kristi Miller-Saunders, [REDACTED] and Gabrielle Malcolm (Ex Officio Secretariat)

**Regrets:** Jay Parsons, James Kristmanson, [REDACTED]

#### **1. Welcome and Introductions**

#### **2. Reviewed Agenda**

- The agenda was approved by the Steering Committee (SC).

#### **3. Reviewed Minutes**

- The minutes from the SC meeting on December 10<sup>th</sup>, 2018 were approved by the SC.

#### **4. Dates for CSAS peer-review meeting**

- SC members did not have any concerns with the addition of a half day on Monday, January 28. The meeting will thus begin at 1PM on Monday, January 28 and go until 5PM on Wednesday, January 30.

#### **5. Update on the venue in Vancouver, BC**

- The CSAS meeting will be held at the Delta Hotels Vancouver Downtown Suites (550 W Hastings Street, Vancouver, BC) in the Mount Pleasant conference room. Details are provided in the CSAS meeting agenda.

#### **6. Review of the CSAS Agenda**

- There was a concern that the allocation of an hour to the discussion of the pathogen paper would not be enough time. It was explained that there is some flexibility in the agenda for the CSAS meeting, and that if more time was needed, there would be no problem allocating more time for the discussion.
- It was clarified that Edmund Peeler and Ted Meyers will be providing written critiques on the respective papers they are reviewing, but will not be attending the meeting in person. Their reviews will be read out-loud at the peer-review meeting.
- Should there be weather related travel interruptions; the addition of a conference line will be investigated.



#### **7. Update on identification of formal reviewers and meeting participants**

- The process for inviting experts according to the SC rankings to either review or participate in the CSAS meeting was presented along with the final listing of who declined and who accepted to participate.
- [REDACTED] provided an update on his request through the First Nations Fisheries Council (FNFC) to identify experts for participating at the CSAS meeting. [REDACTED] were put forward by the council as potential participants with appropriate expertise for the CSAS peer-review meeting. Ranking results of formal reviewers and meeting participants, as reviewed and agreed upon by the SC, resulted in the Department extending an invitation [REDACTED] will be attending the CSAS meeting.

- Two SC members expressed that they believed an exception should have been made [REDACTED]

[REDACTED] It was explained that the process of ranking and inviting formal reviewers and participants, which was agreed upon by the SC, was followed and that exceptions would not be made.

#### **8. Distribution of the working papers**

- The working papers will be sent out shortly after the call.
- It was explained that all meeting participants are expected to read the material and participate in the discussions at the CSAS meeting.
- All meeting participants are encouraged to prepare comments on the working papers and share them at the meeting.
- There are five papers that were published following the previous CSAS peer-review process on IHNV risk assessment. Some of the research documents are used in the PRV risk assessment. A link to these documents will be provided to all meeting participants along with the meeting materials (working papers) and CSAS agenda.
- An update on the status of the research documents from the four bacteria CSAS was provided. Refinements to the documents that had been requested during the last CSAS peer-review meeting are currently being incorporated into the research documents. As agreed upon at the last CSAS, the four bacteria risk assessments will be reviewed by one of the formal reviewers for approval before publication.

#### **9. Next Steps**

- Working papers and the approved CSAS agenda will be distributed.
- CSAS meeting to commence on Monday, January 28 at 1PM (PST).

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** January-16-19 4:41 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re: Chat request

Yes ... yesterday afternoon.

[REDACTED]

> On Jan 16, 2019, at 4:38 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>

> Have they distributed the documents yet? I did not see it in my email?

>

> From: [REDACTED]

> Sent: January 16, 2019 4:27 PM

> To: Miller-Saunders, Kristi

> Subject: Re: Chat request

>

> Just wait ...

>

>

> IMO, the paper on PRV is also somewhat biased. A lot of fairly definitive statements are made based on rather flimsy evidence and in the conclusions the authors make the a priori determination that risk to Sockeye is minimal, despite the fact they did not do a risk assessment.

>

> [REDACTED]

>

>> On Jan 16, 2019, at 4:19 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>>

>> OK, I will get in touch when I am back in the office next week. [REDACTED]

[REDACTED]

>>

>> From: [REDACTED]

>> Sent: January 16, 2019 1:05 PM

>> To: Miller-Saunders, Kristi

>> Subject: Re: Chat request

>>

>> Yes.

s.19(1)

>>

s.21(1)(b)

>> Whenever you are free.

>>

> [REDACTED]

>>  
>>> On Jan 16, 2019, at 12:45 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:  
>>>  
>>> [REDACTED] Can it wait till next week?  
>>>  
>>> From: [REDACTED]  
>>> Sent: January 16, 2019 10:57 AM  
>>> To: Miller-Saunders, Kristi  
>>> Subject: Chat request  
>>>  
>>> Hi Kristi:  
>>>  
>>>  
>>> I am just wondering if we can connect and talk about things. I have some science-related questions to ask.  
>>>  
>>>  
>>> [REDACTED]  
>>>  
>>> [REDACTED]  
>>>  
>>> [REDACTED]  
>>>  
>>> David Suzuki Foundation  
>>>

s.19(1)

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** January-23-19 9:55 AM  
**To:** Malcolm, Gabrielle  
**Cc:** [REDACTED] Struthers, Alistair; Waddington, Zac; Miller-Saunders, Kristi; Gagne, Nellie; 'Bruneau, Nathalie (CFIA/ACIA) (nathalie.bruneau@canada.ca)'; 'myron.roth@gov.bc.ca'; [REDACTED] Olivier, Gilles; 'Craig Stephen'; Parsons, Jay; Burgetz, Ingrid; Kristmanson, James  
**Subject:** Re: DFO PRV Steering Committee Meeting Minutes

Thank you Gabrielle for the minutes.

I would like to point out that in regards to the participation of First Nations representatives ... the comment:

"It was explained that the process of ranking and inviting formal reviewers and participants, which was agreed upon by the SC, was followed and that exceptions would not be made."

This was not the final outcome I heard. I heard Gilles say that the participant selection process is "almost" final and that he could not make a determination that accommodation should/could be made to change the outcome.

He would also not allow a vote of the Steering Committee members on the call to amend the selections despite a request to do so and referred to the "process" of rankings and asserted that that was the process that had been followed ... but I took away that this issue about FN participation would be kicked up the ladder.

Am I wrong in that assumption?

As has been repeatedly mentioned, this is to be a science driven exercise employing the knowledge of experts in the field of salmon biology and Pathogen transmission (specifically with regards to PRV) yet some of the key experts working on this issue here in B.C. are excluded from participating because of a "process" ... a process being adhered to by the Chair (which I should note was not agreed to by all .. I have voiced my opinion on that) without even asking the SC whether that process can be amended. I think that is an important omission from these minutes.

Balance is an important consideration in discussing this critical issue and IMO, there is no balance in the selection of participants.

IMO, there are people on the list of participants that have been selected on the basis of name recognition and availability only rather than expertise.

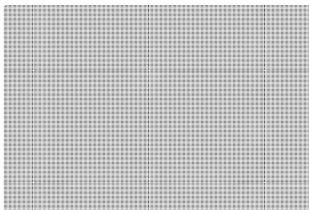
I have to question why that is ... and I don't want to name names ... Why are we rejecting experienced, published scientists in favour of people that have no direct connection to, or influence on, the issue? It makes no sense.

I would point out that the Independent Expert Panel on Aquaculture Science has addressed the issue of how Fisheries and Oceans Canada has approached the issue of risk management in relation to finfish aquaculture, how the relevant

science is reviewed and interpreted and how peer-review is conducted, and on these matters they were quite critical. They made many recommendations for change

<https://www.ic.gc.ca/eic/site/052.nsf/eng/00011.html>

One would expect that, in the face of such criticism from a panel of experts, there would be an effort to try to address the short-comings addressed by the Panel, but I am not seeing movement in that direction in regards to this process.



On Jan 16, 2019, at 11:37 AM, Malcolm, Gabrielle <[Gabrielle.Malcolm@dfo-mpo.gc.ca](mailto:Gabrielle.Malcolm@dfo-mpo.gc.ca)> wrote:

Dear Steering Committee members,

Please find attached the Minutes from yesterday's call.

If you have any questions, concerns or comments about the Minutes, please do not hesitate to contact me.

Kind regards,

**Gabrielle Malcolm**

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[gabrielle.malcolm@dfo-mpo.gc.ca](mailto:gabrielle.malcolm@dfo-mpo.gc.ca) / T: 613-949-7466

Conseillère des sciences, sciences de réglementation de l'aquaculture, Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques

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<image001.jpg>

<SC Meeting Minutes - January 15, 2019 .docx>

s.19(1)

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** January-29-19 7:18 AM  
**To:** [REDACTED] Miller-Saunders, Kristi; [REDACTED] Parsons, Jay; Johnson, Stewart; Garver, Kyle  
**Cc:** [REDACTED]  
**Subject:** Response to Laurin et al post

Hello

Two days before your CSAS Review began, I note that a post appeared on the PLoS One comment board which takes issue with Morton et al 2017. Morton et al 2017 provides results on the distribution of PRV infection in wild BC salmon coast wide and in farmed salmon and steelhead.

<https://journals.plos.org/plosone/article/comment?id=10.1371/annotation/37c3e4ea-cd66-4a67-a548-67bac60a7e41>

This post by Drs. Laurin, Gardner and Christensen contains the same material rejected by PLoS One from anonymous “readers” for publication as a “Formal Comment”. Now that this material is public as a non-reviewed post on the journal website, I have uploaded our response and I want to be certain that you have the opportunity to review our response.

<https://journals.plos.org/plosone/article/comments?id=10.1371/journal.pone.0188793>

Further to this, I want to ensure that your CSAS considers the Formal Comment [REDACTED] published in PLoS One on Siah et al 2015, which led to their publication of a “correction” identifying their conclusion that they had ruled out recent introduction of PRV to BC as “overstated”. Our Formal Comment on this topic provides further evidence that a Norwegian strain of PRV is widespread in BC.

**Siah et al Correction:** <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0164926>

**Formal Comment on Siah et al:** <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0188690>

In my view, the unpublished post by Laurin et al highlights why it was important that this CSAS review include members of all the research teams publishing on PRV in BC, particularly scientists recommended by First Nations. However, I want to make sure that you have the most up-to-date material for your review, as reporting on the impact of this virus continues to become apparent in new papers being published at frequent interval.

[REDACTED]

s.19(1)

## Miller-Saunders, Kristi

---

**From:** Miller-Saunders, Kristi  
**Sent:** February-01-19 4:07 PM  
**To:** Weber, Lily  
**Subject:** RE: PRV-point of contact

In terms of the coding to pay for travel costs, how do we deal with that?

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

**From:** Weber, Lily  
**Sent:** January-29-19 8:34 AM  
**To:** Olivier, Gilles; cstephen@cwahc-rcsf.ca; Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair; Gagne, Nellie; Nathalie.N.Bruneau@inspection.gc.ca; Myron.Roth@gov.bc.ca; [REDACTED] Miller-Saunders, Kristi; [REDACTED] espen.rimstad@nmbu.no; niven@vet.dtu.dk; mark.powell@hi.no; iagardner@upei.ca; Garver, Kyle; Polinski, Mark; Weber, Lily; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED] tony.farrell@ubc.ca; [REDACTED] Gary.Marty@gov.bc.ca; [REDACTED] Boily, France; Garver, Kyle  
**Cc:** Parsons, Jay  
**Subject:** PRV-point of contact

Good morning CSAS participants,

Thank you for your participation thus far. As you may already know Gabby has moved on to other endeavors. I will be your point of contact for this process.

Please do not hesitate if you have any questions or concerns.

Thank you,  
**Lily Weber**

Science Advisor, Aquaculture, Biotechnology and Aquatic Animal Health Science Branch  
Fisheries and Oceans Canada / Government of Canada  
[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca) / Tel: [REDACTED]

Conseillère scientifique, Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques  
Pêches et Océans Canada / Gouvernement du Canada  
[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca) / Tel: [REDACTED]



Government  
of Canada

Gouvernement  
du Canada

Canada

s.16(2)(c)

s.19(1)

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** February-06-19 5:25 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re: PRV decision

OK, thanks Kristi.

On Wed, Feb 6, 2019 at 8:23 PM Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)> wrote:

Not sure how much I can add [REDACTED] It is not clear at all what the Minister will do with this ruling and it looks like the judge left many option open to him. I am not sure why the news media came out saying that the judge said the no testing policy for PRV had to be changed. In my reading, she said that the bar for the level of risk required to initiate action, being at the level of extirpation of a conservation unit, was too high and not consistent with the precautionary principle or with the wild salmon policy.

I can say that Cermaq and Marine Harvest, without the requirement from the regulator, have already taken the initiative over the past few years to produce PRV-free smolts, and with good success. So it appears that it is not an impossible situation after all.

Given this is a policy issue, rather than a science one, I really can't speak to it further than that.

Kristi Miller

---

**From:** [REDACTED]  
**Sent:** February 6, 2019 3:57 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** PRV decision

Kristi -- Somebody suggested you would be a very good person to chat with about this story that ran two days ago. A big salmon farmer tells us that it is going to be very difficult for his industry in BC to deal with this ruling as so many salmon have PRV in the region. I'm on the East Coast (in Virginia) but free tonight or tomorrow anytime to chat.

<https://www.thestar.com/vancouver/2019/02/04/federal-court-overturns-controversial-salmon-farm-policy.html>

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Sincerely,

[REDACTED]  
Undercurrent News  
[REDACTED]

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Sincerely,



## Undercurrent News

s.19(1)

No further information has been removed or severed from this page

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** February-06-19 5:36 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re: CSAS report highly anticipated

There's a press conference called for tomorrow.

The Co-chairs of this recent CSAS review ... will give a media technical briefing to discuss the peer-review results.

It is for accredited media only ..

My understanding is we were sworn to confidentiality until the SAR and the working papers were finalized.

I have been on many CSAS reviews and this has never happened.

Don't know what they will or will not say ... just wanted to give you a heads-up.

[REDACTED]

[REDACTED]

> On Feb 6, 2019, at 5:13 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>

> Not sure which aspect you think is the bad news (or I should say the "surprising" news). Seems like more of the same to me.

>

> From: [REDACTED]

> Sent: February 6, 2019 1:21 PM

> To: Miller-Saunders, Kristi

> Subject: RE: CSAS report highly anticipated

>

> Here is the text of the story minus the pictures.

s.19(1)

s.68(a)

> Available at

> <https://www.undercurrentnews.com/2019/02/06/british-columbia-prv-report-to-land-at-pivotal-time-for-salmon-farms/>

>

**Page 127**

**is withheld pursuant to section  
est retenue en vertu de l'article**

**68(a)**

**of the Access to Information Act  
de la Loi sur l'accès à l'information**

> -----Original Message-----

> From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]

> Sent: February-06-19 1:14 PM

> To: [REDACTED]

> Subject: RE: CSAS report highly anticipated

>

> I am not a subscriber. Can you sent article?

>

> From: [REDACTED]

> Sent: February 6, 2019 9:40 AM

> To: Miller-Saunders, Kristi

> Subject: CSAS report highly anticipated

>

> Not good news.

>

> [https://www.undercurrentnews.com/2019/02/06/british-columbia-prv-report-to-land-at-pivotal-time-for-salmon-farms/?utm\\_source=Undercurrent+News+Alerts&utm\\_campaign=767278d8f6-](https://www.undercurrentnews.com/2019/02/06/british-columbia-prv-report-to-land-at-pivotal-time-for-salmon-farms/?utm_source=Undercurrent+News+Alerts&utm_campaign=767278d8f6-Americas_briefing_Feb_06_2019&utm_medium=email&utm_term=0_feb55e2e23-767278d8f6-92479985)

> [Americas\\_briefing\\_Feb\\_06\\_2019&utm\\_medium=email&utm\\_term=0\\_feb55e2e23-767278d8f6-92479985](https://www.undercurrentnews.com/2019/02/06/british-columbia-prv-report-to-land-at-pivotal-time-for-salmon-farms/?utm_source=Undercurrent+News+Alerts&utm_campaign=767278d8f6-Americas_briefing_Feb_06_2019&utm_medium=email&utm_term=0_feb55e2e23-767278d8f6-92479985)

>

> [REDACTED]

s.19(1)

s.68(a)

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** February-06-19 8:15 PM  
**To:** Miller-Saunders, Kristi; [REDACTED]  
**Subject:** Fwd: CSAS PRV communications  
**Attachments:** image001.png; ATT00001.htm; SUMMARY BULLETS.pdf; ATT00002.htm

They are going down a road I thought they would not travel.

This is unprecedented in the history of CSAS.

And the instruction to participants is .. stick to the bullet points if contacted by media.



Begin forwarded message:

**From:** "Parsons, Jay" <Jay.Parsons@dfo-mpo.gc.ca>  
**Date:** February 6, 2019 at 7:07:29 PM PST  
**To:** "Olivier, Gilles" <Gilles.Olivier@dfo-mpo.gc.ca>, "cstephen@cwhc-rcsf.ca" <cstephen@cwhc-rcsf.ca>, "Burgetz, Ingrid" <Ingrid.Burgetz@dfo-mpo.gc.ca>, "Waddington, Zac" <Zac.Waddington@dfo-mpo.gc.ca>, "Struthers, Alistair" <Alistair.Struthers@dfo-mpo.gc.ca>, "Gagne, Nellie" <Nellie.Gagne@dfo-mpo.gc.ca>, "Nathalie.N.Bruneau@inspection.gc.ca" <Nathalie.N.Bruneau@inspection.gc.ca>, "Myron.Roth@gov.bc.ca" <Myron.Roth@gov.bc.ca>, [REDACTED], "Miller-Saunders, Kristi" <Kristi.Saunders@dfo-mpo.gc.ca>, [REDACTED], "espen.rimstad@nmbu.no" <espen.rimstad@nmbu.no>, "niven@vet.dtu.dk" <niven@vet.dtu.dk>, "mark.powell@hi.no" <mark.powell@hi.no>, "iagardner@upei.ca" <iagardner@upei.ca>, "Garver, Kyle" <Kyle.Garver@dfo-mpo.gc.ca>, "Polinski, Mark" <Mark.Polinski@dfo-mpo.gc.ca>, "Weber, Lily" <Lily.Weber@dfo-mpo.gc.ca>, "Mimeault, Caroline" <Caroline.Mimeault@dfo-mpo.gc.ca>, "Holt, Kendra" <Kendra.Holt@dfo-mpo.gc.ca>, "Johnson, Stewart" <Stewart.Johnson@dfo-mpo.gc.ca>, "Jones, Simon" <Simon.Jones@dfo-mpo.gc.ca>, [REDACTED], [REDACTED], "tony.farrell@ubc.ca" <tony.farrell@ubc.ca>, [REDACTED], "Gary.Marty@gov.bc.ca" <Gary.Marty@gov.bc.ca>, [REDACTED], [REDACTED], "Boily, France" <France.Boily@dfo-mpo.gc.ca>  
**Subject:** CSAS PRV communications

Colleagues,

s.19(1)

I want to provide a quick update following the PRV CSAS meeting from last week. We are finalizing the other sections of the SAR (Background, Analysis and Other Considerations) and we should be able to distribute this to you for your review by next week.

As well, given the considerable interest on PRV in British Columbia and in the spirit of transparency, there is much interest in having the Department communicate some information on this process. To that end, the Department will issue a News Release tomorrow on the high level findings of the CSAS meeting. The News Release will be a plain language summary of the key findings and it will be consistent with the wording of the agreed-to summary bullets that we developed at the meeting. In addition, there will be a media technical briefing tomorrow by the Co-chairs on the key findings of the CSAS meeting (i.e., the summary bullets).

I recognize that as a result of the News Release and the media technical briefing, media may approach some of you with questions on the process and / or findings. If approached by the media, we ask that any discussion on the results of the peer-review be limited to our agreed-on summary bullets, at least until the full SAR is approved by everyone. As a reminder, I have attached the summary bullets as discussed at the meeting.

Please let me know if there are any questions or concerns.

Jay

**Jay Parsons, PhD**

**Director**

Aquaculture, Biotechnology and Aquatic Animal Health Sciences Branch  
Fisheries and Oceans Canada / Government of Canada  
200 Kent Street, Stn 12E239 Ottawa, ON Canada K1A 0E6  
[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca) / Tel. 613-990-0278

**Directeur**

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[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca) / Tél. 613-990-0278

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** February-07-19 7:09 AM  
**To:** Farrell, Anthony  
**Cc:** Parsons, Jay; Olivier, Gilles; cstephen@cwhc-rcsf.ca; Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair; Gagne, Nellie; Nathalie.N.Bruneau@inspection.gc.ca; Myron.Roth@gov.bc.ca; [REDACTED] Miller-Saunders, Kristi; [REDACTED] espen.rimstad@nmbu.no; niven@vet.dtu.dk; mark.powell@hi.no; iagardner@upei.ca; Garver, Kyle; Polinski, Mark; Weber, Lily; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED]  
[REDACTED] Gary.Marty@gov.bc.ca; [REDACTED] Boily, France  
**Subject:** Re: CSAS PRV communications

[REDACTED] (CTV) and [REDACTED] (Global) are apprised and will be on this call (assuming they are accepted/allowed).

[REDACTED]

On Feb 7, 2019, at 5:03 AM, Farrell, Anthony <tony.farrell@ubc.ca> wrote:

Jay

Great to hear that DFO is moving swiftly and with transparency in terms of public dissemination.

Best

Tony

**From:** "Parsons, Jay" <Jay.Parsons@dfo-mpo.gc.ca>  
**Date:** Wednesday, February 6, 2019 at 7:08 PM  
**To:** "Olivier, Gilles" <Gilles.Olivier@dfo-mpo.gc.ca>, "cstephen@cwhc-rcsf.ca" <cstephen@cwhc-rcsf.ca>, "Burgetz, Ingrid" <Ingrid.Burgetz@dfo-mpo.gc.ca>, "Waddington, Zac" <Zac.Waddington@dfo-mpo.gc.ca>, "Struthers, Alistair" <Alistair.Struthers@dfo-mpo.gc.ca>, "Gagne, Nellie" <Nellie.Gagne@dfo-mpo.gc.ca>, "Nathalie.N.Bruneau@inspection.gc.ca" <Nathalie.N.Bruneau@inspection.gc.ca>, Myron Roth <Myron.Roth@gov.bc.ca>, [REDACTED] Kristi Miller-Saunders <Kristi.Saunders@dfo-mpo.gc.ca>, [REDACTED] "espen.rimstad@nmbu.no" <espen.rimstad@nmbu.no>, "niven@vet.dtu.dk" <niven@vet.dtu.dk>, "mark.powell@hi.no" <mark.powell@hi.no>, "iagardner@upei.ca" <iagardner@upei.ca>, Kyle Garver <Kyle.Garver@dfo-mpo.gc.ca>, "Polinski, Mark" <Mark.Polinski@dfo-mpo.gc.ca>, "Weber, Lily" <Lily.Weber@dfo-mpo.gc.ca>, "Mimeault, Caroline" <Caroline.Mimeault@dfo-

mpo.gc.ca), "Holt, Kendra" <[Kendra.Holt@dfo-mpo.gc.ca](mailto:Kendra.Holt@dfo-mpo.gc.ca)>, "Johnson, Stewart"  
<[Stewart.Johnson@dfo-mpo.gc.ca](mailto:Stewart.Johnson@dfo-mpo.gc.ca)>, Simon Jones <[Simon.Jones@dfo-mpo.gc.ca](mailto:Simon.Jones@dfo-mpo.gc.ca)>,  
[REDACTED]

"Farrell, Anthony" <[tony.farrell@ubc.ca](mailto:tony.farrell@ubc.ca)>,  
[REDACTED]

"Gary.Marty@gov.bc.ca"

<[Gary.Marty@gov.bc.ca](mailto:Gary.Marty@gov.bc.ca)>, "[REDACTED]"

, "Boily, France" <[France.Boily@dfo-mpo.gc.ca](mailto:France.Boily@dfo-mpo.gc.ca)>

**Subject:** CSAS PRV communications

Colleagues,

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As well, given the considerable interest on PRV in British Columbia and in the spirit of transparency, there is much interest in having the Department communicate some information on this process. To that end, the Department will issue a News Release tomorrow on the high level findings of the CSAS meeting. The News Release will be a plain language summary of the key findings and it will be consistent with the wording of the agreed-to summary bullets that we developed at the meeting. In addition, there will be a media technical briefing tomorrow by the Co-chairs on the key findings of the CSAS meeting (i.e., the summary bullets).

I recognize that as a result of the News Release and the media technical briefing, media may approach some of you with questions on the process and / or findings. If approached by the media, we ask that any discussion on the results of the peer-review be limited to our agreed-on summary bullets, at least until the full SAR is approved by everyone. As a reminder, I have attached the summary bullets as discussed at the meeting.

Please let me know if there are any questions or concerns.

Jay

**Jay Parsons, PhD**

**Director**

Aquaculture, Biotechnology and Aquatic Animal Health Sciences Branch  
Fisheries and Oceans Canada / Government of Canada  
200 Kent Street, Stn 12E239 Ottawa, ON Canada K1A 0E6  
[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca) / Tel. 613-990-0278

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[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca) / Tél. 613-990-0278

<image001.png>

s.19(1)



## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** February-07-19 9:26 AM  
**To:** Parsons, Jay  
**Cc:** Olivier, Gilles; cstephen@cwht-rcsf.ca; Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair; Gagne, Nellie; Nathalie.N.Bruneau@inspection.gc.ca; Myron.Roth@gov.bc.ca; [REDACTED] Miller-Saunders, Kristi; [REDACTED] espen.rimstad@nmbu.no; niven@vet.dtu.dk; mark.powell@hi.no; iagardner@upei.ca; Garver, Kyle; Polinski, Mark; Weber, Lily; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED] tony.farrell@ubc.ca; [REDACTED] Gary.Marty@gov.bc.ca; [REDACTED] Boily, France  
**Subject:** Re: CSAS PRV communications

Jay,

I'm writing to express our grave concern re. your plans to proceed with an 11am PT media briefing this morning.

In terms of our participation in CSAS processes over the last couple of decades, this approach (i.e., briefing media on a process that is incomplete, with 6+ weeks of work remaining) - without providing any sort of documentation for CSAS participants, media, etc. to review in advance - is unprecedented and unacceptable.

We have long been trusted participants in CSAS processes, and this - simply put - is not what we signed up for.

**Our recommendation for you at this stage is to cancel today's media briefing.** Otherwise, we will be put in an impossible position, and will speak to media about the flaws in this process.

We appreciate the "spirit of transparency" mentioned in your memo, but not at the expense of derailing DFO's primary science-based peer review process.

Thank you for your time and consideration – we await your response.

s.19(1)

Best,

On Feb 6, 2019, at 7:07 PM, Parsons, Jay <[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca)> wrote:

Colleagues,

I want to provide a quick update following the PRV CSAS meeting from last week. We are finalizing the other sections of the SAR (Background, Analysis and Other Considerations) and we should be able to distribute this to you for your review by next week.

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Jay

**Jay Parsons, PhD**

**Director**

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[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca) / Tel. 613-990-0278

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[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca) / Tél. 613-990-0278

<image001.png>

s.19(1)

<SUMMARY BULLETS.pdf>

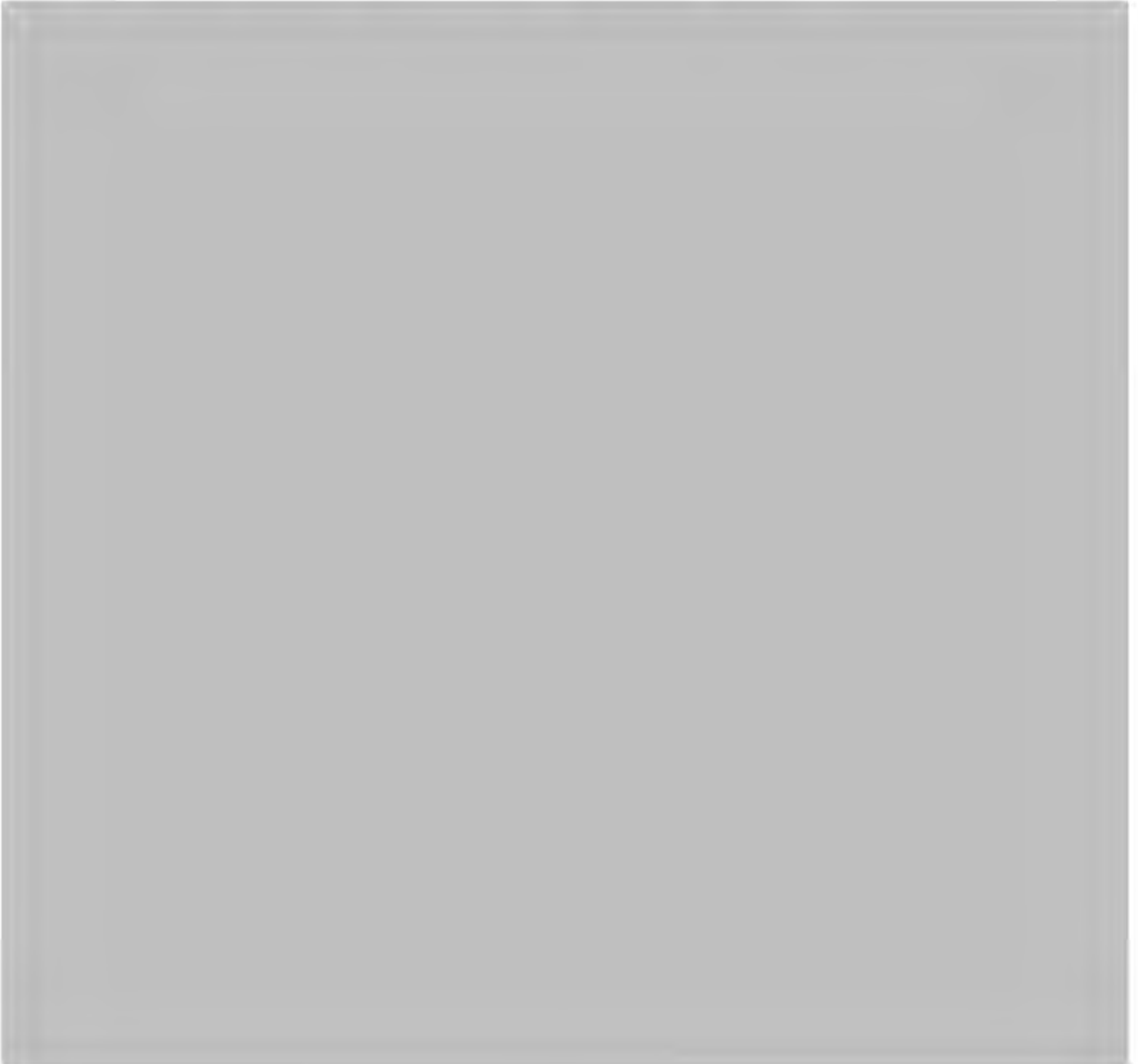
No information has been removed or severed from this page

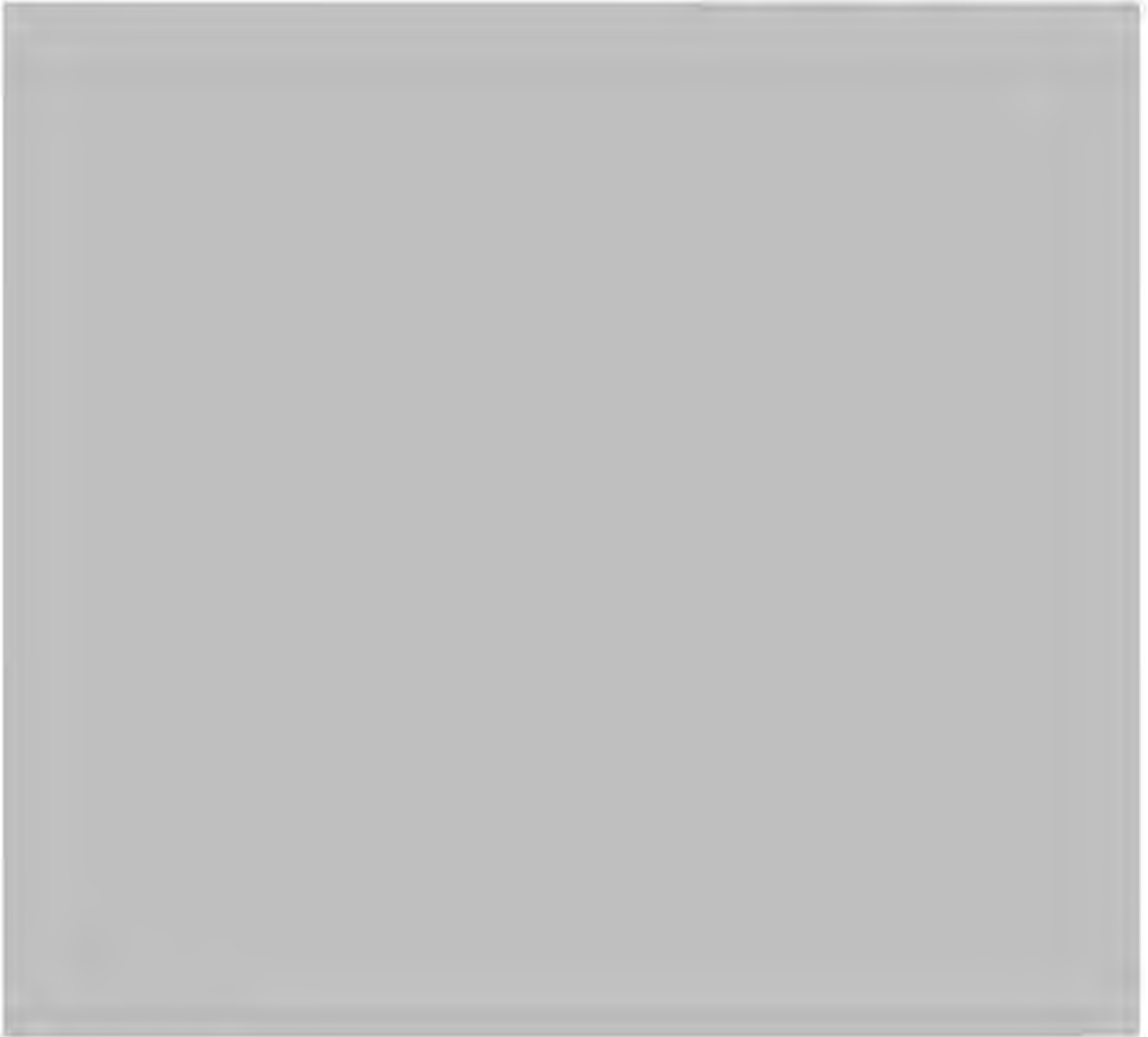
**Miller-Saunders, Kristi**

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**From:** [REDACTED]  
**Sent:** February-07-19 7:02 PM  
**To:** [REDACTED] Miller-Saunders, Kristi  
**Subject:** Undercurrent News

Available at <https://www.undercurrentnews.com/2019/02/07/british-columbia-prv-report-winds-minimal-risk-to-wild-sockeye/>





[Redacted]

s.19(1)  
s.68(a)

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** February-07-19 7:24 PM  
**To:** [REDACTED]  
**Cc:** Miller-Saunders, Kristi  
**Subject:** Re: PRV media release

Agree [REDACTED]

I just wish I was not the only voice speaking out about this.

Senior DFO staff in Ottawa are painting this as Environmentalist sour grapes ... claiming we are only complaining because we did not get the outcome we wanted!!!

Really?

[REDACTED]

> On Feb 7, 2019, at 1:39 PM, [REDACTED] wrote:

>

> They have again been very selective in what they reported AGAIN. Is a rebuttal merited?

>

> They did exactly what I said ... use half the conclusion omitting the uncertainties! That was not the consensus statement. [REDACTED]

>

>

> [REDACTED]

s.19(1)

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** February-12-19 10:43 AM  
**To:** Olivier, Gilles; cstephen@cwahc-rcsf.ca; Burgetz, Ingrid; Waddington, Zac; [REDACTED]  
Struthers, Alistair; Gagne, Nellie; Myron.Roth@gov.bc.ca; Miller-Saunders, Kristi;  
[REDACTED] niven@vet.dtu.dk; mark.powell@hi.no; Espen Rimstad; [REDACTED]  
**Subject:** Minimal Risk

Dear PRV CSAS Panel Experts:

I am writing to confirm that all of you are in agreement with the statement attributed to you in the February 7, 2019 Fisheries and Oceans Canada press release:

"The scientific experts who peer reviewed the data and risk assessment reached a consensus that the risk to Fraser River sockeye salmon due to PRV is minimal."

I request the scientific evidence that you based your conclusions on.

Thank you,

[REDACTED]

s.19(1)

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** February-13-19 10:15 AM  
**To:** Miller-Saunders, Kristi  
**Cc:** [REDACTED]  
**Subject:** RE: CSAS press briefing

Thanks, Kristi

As a first step, we at DSF need to prepare a very science-based document commenting on the issues in this CSAS around uncertainty, lack of evidence and study design in relation to the conclusions put forward in the DFO press briefing. We pulled out a lot of stops contacting high-ranking DFO to try and get this press technical briefing stopped. We got quite a strong push back from people like Alexis McIntyre that we were having "sour grapes," i.e. just an ENGO complaining that we did not get our way. It is very important to me that we counter this message with facts and also try and show where the process could be objectively better.

Ideally, we would write such a letter and deliver it privately in a way that made it possible for other participants in the peer review or paper review process to sign on, or deliver the same message.

[REDACTED] drafting that letter. Without committing to support it / sign it, would you at least be willing to review our draft and let us know if we are making a well supported argument?

I note that [REDACTED] also reaching out to the CSAS committee - I have let her know about our idea to start by trying to push from the inside with this sort of "no publicity, science-based approach" that might appeal to some of the review committee members who are uncomfortable with DFO's actions on this, but reluctant to publically criticise. Obviously, that approach might not work, so I am glad she is doing her work, as well.

Interested in your thoughts.

Cheers

[REDACTED]  
-----Original Message-----

**From:** Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]  
**Sent:** Thursday, 7 February, 2019 14:07  
**To:** [REDACTED]  
**Cc:** [REDACTED]  
**Subject:** RE: CSAS press briefing

Sorry I only just got to reading my email and seeing your message and u see [REDACTED] that they went ahead with a very biased presentation of the findings. I have not had a chance to read what was said but absolutely share your concerns and I don't know why they would take such an unprecedented approach. [REDACTED] I would imagine media may want to confirm with participants their interpretation of the results.

What are u thinking in terms of response?  
Kristi

s.19(1)  
s.21(1)(b)

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**From:** [REDACTED]



Sent: February 7, 2019 10:41 AM  
To: Miller-Saunders, Kristi; Miller-Saunders, Kristi  
Cc: [REDACTED]  
Subject: CSAS press briefing

Kristi,

If there is any chance you can also urge Jay Parsons or anyone with pull at DFO to cancel or postpone this press briefing we would really appreciate it. We were really hoping to be able to continue to work on contextualising and getting some better nuance into the final CSAS proceedings over the last weeks of revision and editing and none of us has ever seen this happen in a CSAS process, before.

This is a bad idea for DFO, too. It is actually going to bring more attention to PRV and create a bunch of "DFO science is manipulated and untrustworthy" commentary in the media. As unhappy with this particular CSAS process as we are, we don't want to see the whole peer review process get dragged into a media storm.

Is there any chance other CSAS participants also feel uncomfortable being put in a corner like this? Savvy media have already reached out to us asking why DFO is doing this, where are the usual technical briefing documents and why does this seem so fishy? We have not responded but will not really have any choice if this press conference goes ahead. A simple "the press conference has been postponed" from DFO Communications would stop the whole mess and give us all time to properly complete the CSAS process.

Thanks in advance for any advice or assistance.

[REDACTED]

[REDACTED]

Western Canada

[REDACTED]

+1 (604) 732-4228, ext [REDACTED]

219-2211 West 4th Avenue  
Vancouver, BC V6K 4S2  
CANADA

s.19(1)

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** February-13-19 3:52 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re: Quick question about PRV

Thanks for getting back to me. [REDACTED]

Just confirming that there has been no change in your finding that PRV can cause illness in chinook Salmon. And that you suspect there is a link between the virus and the salmon farms.  
But you don't have to respond. I've pretty well finish the story.

Thanks once again,  
[REDACTED]

[REDACTED]  
Reporter  
Global BC News  
[REDACTED]

> On Feb 13, 2019, at 3:43 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>

> You should discuss this [REDACTED] He is well aware of this situation [REDACTED]  
[REDACTED]

>

> Kristi Miller-Saunders, PhD  
> Head, Molecular Genetics  
> Pacific Biological Station  
> 3190 Hammond Bay Rd  
> Nanaimo BC V9T 6N7  
> 250-756-7155  
> Kristi.Saunders@dfo-mpo.gc.ca

>

>

> -----Original Message-----

> From: [REDACTED]  
> Sent: February-13-19 1:41 PM  
> To: Miller-Saunders, Kristi  
> Subject: Quick question about PRV

>

> Hi Kristi

> I just interviewed Jonathan Wilkinson about PRV. When I cited your study on the effects of PRV on wild chinook salmon he said it wasn't true. What am I to make of that?

> s.19(1)

> [REDACTED] s.21(1)(b)

>

> Reporter  
> Global BC News

s.19(1)

> [REDACTED]  
> [REDACTED]  
> [REDACTED]  
> [REDACTED]  
> [REDACTED]  
> [REDACTED]

No further information has been removed or severed from this page

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** February-14-19 8:32 AM  
**To:** [REDACTED]  
**Cc:** Simons, Fiona; [REDACTED] Miller-Saunders, Kristi; DiCicco, Emiliano  
**Subject:** Re: Quick question about PRV

Thank you so much for this information.

I would like to do a follow up on this but don't expect Kristi Miller to go on camera. Would others be willing to speak? I have the minister on tape denying the science. He continually referred to the sockeye study even when I specifically mentioned Chinook.

By the way I am not able to do a story on this today. But I'm wide open any other day it is convenient.

Thanks,

[REDACTED]

[REDACTED]

Reporter  
Global BC News

[REDACTED]

> On Feb 14, 2019, at 7:05 AM, [REDACTED] wrote:

>

> Hi [REDACTED]... It is extremely disappointing to hear that the Minister has been advised to not believe the science on PRV and Chinook salmon that was published almost a year ago (citation below). Particularly since we have just completed a scientific review process (CSAS review on PRV that was recently in the media also). The Di Cicco paper was cited in that review and never opposed on its content (other than a Chinook salmon is not a Sockeye salmon and so was not specifically considered). What is most troubling is that the Minister's advice must have been based on an internal DFO Science review that was conducted last spring through the Centre for Science Advice, Rapid Response process:

>

> <https://protect2.fireeye.com/url?k=be5870ad-e23d41a5-be5c14c0-0cc47ad9c1d0-53fc1ddf7e25135d&u=http://www.dfo-mpo.gc.ca/csas-sccs/process-processus/srp-prs-eng.htm>

>

> That review was responding to a request for advice from DFO's Aquaculture Management Division concerning this specific paper and requesting advice on whether the Division should alter any protocols in response to these new findings. We have absolutely no concern for a regulatory group asking for science advice and seeking a fast reply given their responsibilities for farm inspections and audits. However we had great concern for how this internal peer review was conducted and we have noted our concerns to DFO. Particularly that the review was conducted with complete exclusion of the primary researchers ... essentially holding court without notifying the defendants.

>

> The Di Cicco et al. paper received thorough peer review (3 journal reviewers) and has NOT received any subsequent correspondence regarding concerns about procedures or conclusions. In the formal science review process when other researchers have concerns, journals provide opportunities for comment and also allow the authors to provide a reply. This provides for open dialogue and transparency in the process.

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>  
> If there is scientific concern for this paper, and we have openly declared this, it is that the study is NOT a direct challenge study where uninfected fish would be exposed to the virus and subsequently examine for direct causation of any effect (lesions, behavioural changes, poor growth, etc.). The Di Cicco study was based on audit samples collected by DFO and then underwent the usual screening for presence of disease/pathogens. The Di Cicco study used fish tissue that had already been collected and examined, and directly tested for the presence of Piscine Reovirus via immunochemistry. A testing procedure that exposes the presence of the virus using chemical stains that are specific to only PRV and can then be observed in each specific tissue examined. While not a challenge study, there was complete coherence between tissue lesions and PRV in these Chinook salmon. Further, the study examined the developmental pathway of HSMI and jaundice/anemia associated with PRV-1 in farmed Atlantic and chinook salmon and was able to then conclude that the same virus of concern with Atlantic salmon does have associated effects of concern in a Pacific salmon ... but not the same effects.

>  
> To hear that the Minister stated that this study and outcome "wasn't true" is truly disappointing ... the presence of PRV was real and observable. If others advising the Minister wish to disregard this study, that is their decision and opinion. Our advice would certainly be different and is based on empirical observations that should be disproved through science and not simply disregarded when our wild Pacific salmon are involved! Disappointing for sure.

>  
> Di Cicco, E., et al. 2018. The same strain of Piscine orthoreovirus (PRV) is involved with the development of different, but related, diseases in Atlantic and Pacific Salmon in British Columbia. FACETS 3: 599-641.

>  
> Sorry, not as quick an answer as you may have been hoping for.

>  
> [REDACTED]  
> [REDACTED] Pacific Salmon Foundation,  
> 300 – 1682 West 7th Avenue, Vancouver, BC V6J 4S6  
> 604-664-7664 (office phone)  
> 604-664-7665 (office fax)  
> [REDACTED] (cell)

s.19(1)

s.21(1)(b)

> -----Original Message-----

> From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]  
> Sent: February 13, 2019 3:44 PM  
> To: [REDACTED]  
> Cc: [REDACTED]  
> Subject: RE: Quick question about PRV

>  
> You should discuss this [REDACTED] He is well aware of this situation [REDACTED]

>  
> Kristi Miller-Saunders, PhD  
> Head, Molecular Genetics  
> Pacific Biological Station  
> 3190 Hammond Bay Rd  
> Nanaimo BC V9T 6N7  
> 250-756-7155  
> Kristi.Saunders@dfo-mpo.gc.ca

>  
>  
> -----Original Message-----

> From: [REDACTED]  
> Sent: February-13-19 1:41 PM

> To: Miller-Saunders, Kristi  
> Subject: Quick question about PRV  
>  
> Hi Kristi  
> I just interviewed Jonathan Wilkinson about PRV. When I cited your study on the effects of PRV on wild chinook salmon he said it wasn't true. What am I to make of that?  
>  
> [REDACTED]  
>  
> [REDACTED]  
> Reporter  
> Global BC News  
> [REDACTED]  
>  
>

s.19(1)

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** February-14-19 11:23 AM  
**To:** Withler, Ruth  
**Subject:** FW: requires input by 10:30 TODAY  
**Attachments:** CAAquaculture 2019Feb11600.docx; AquaTacticsTableFeb11600.docx

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

**From:** MacDougall, Lesley  
**Sent:** February-14-19 9:34 AM  
**To:** Lowe, Geoff; Garver, Kyle; Miller-Saunders, Kristi; Kreiberg, Henrik; Jones, Simon  
**Subject:** requires input by 10:30 TODAY

First – apologies (as usual) for the impossible timeline. I didn't see this email earlier today: we're being asked to send any comments we have on this document to Catherine by 10:30.

So, please read through, we won't bother trying to collate from our division with such short time to respond, just send comments directly back to Catherine.

Thanks  
Lesley

**From:** Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>  
**Sent:** February-14-19 8:05 AM  
**To:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>; Kennedy, Eddy <Eddy.Kennedy@dfo-mpo.gc.ca>  
**Cc:** Dickie, Catherine <Catherine.Dickie@dfo-mpo.gc.ca>  
**Subject:** Fw: For RDG approval

I just exchanged emails with Allison about this and seems there is still opportunity for us to input even though we were not reached out to directly. I suggest you review - send any comments you have to Catherine who can collate our feedback and send to Allison. Would ask that you get your input to Catherine if at all possible by 10:30am. This should allow her to send a consolidated response to Allison by no later than 11 am.

Carmel

Sent from my BlackBerry 10 smartphone on the Rogers network.

**From:** Webb, Allison <Allison.Webb@dfo-mpo.gc.ca>  
**Sent:** Wednesday, February 13, 2019 22:51  
**To:** Lowe, Carmel; MacDougall, Lesley

**Subject:** Fw: For RDG approval

I only glanced at this this eve to find it's mostly all science aqua Comms so thought that you both might wish to be aware abd/or comments t if you haven't already seen it. This is the first time I became aware of this. Our team was not involved in developing this. Thx.

Sent from my BlackBerry 10 smartphone on the Bell network.

**From:** Webb, Allison <[Allison.Webb@dfo-mpo.gc.ca](mailto:Allison.Webb@dfo-mpo.gc.ca)>  
**Sent:** Wednesday, February 13, 2019 9:39 AM  
**To:** McCorquodale, Brenda; Patirana, Anoma; Paylor, Adrienne  
**Subject:** FW: For RDG approval

Allison Webb, Director / Directrice  
Aquaculture Management / Gestion de l'aquaculture

Fisheries Management Branch / Direction de la gestion des pêches

Fisheries and Oceans Canada / Pêches et Océans Canada

200 - 401 Burrard St / Rue Burrard, Vancouver BC / C.B. V6C 3S4 Canada

604-666-7009

[Allison.webb@dfo-mpo.gc.ca](mailto:Allison.webb@dfo-mpo.gc.ca)

**From:** Rainer, Michelle <[Michelle.Rainer@dfo-mpo.gc.ca](mailto:Michelle.Rainer@dfo-mpo.gc.ca)>  
**Sent:** Tuesday, February 12, 2019 1:21 PM  
**To:** Webb, Allison <[Allison.Webb@dfo-mpo.gc.ca](mailto:Allison.Webb@dfo-mpo.gc.ca)>  
**Subject:** Fw: For RDG approval

Hi alison,  
Did you have any comments o this?  
Thanks,  
Michelle

Sent from my BlackBerry 10 smartphone on the Rogers network.

**From:** Girouard, Louise <[Louise.Girouard@dfo-mpo.gc.ca](mailto:Louise.Girouard@dfo-mpo.gc.ca)>  
**Sent:** Monday, February 11, 2019 8:01 AM  
**To:** Rainer, Michelle; Jenkins, Phil  
**Subject:** FW: For RDG approval

Thanks Phil – will do.  
L

**From:** Jenkins, Phil <[Phil.Jenkins@dfo-mpo.gc.ca](mailto:Phil.Jenkins@dfo-mpo.gc.ca)>  
**Sent:** Monday, February 11, 2019 7:28 AM  
**To:** Girouard, Louise <[Louise.Girouard@dfo-mpo.gc.ca](mailto:Louise.Girouard@dfo-mpo.gc.ca)>  
**Cc:** Quinn, Caroline <[Caroline.Quinn@dfo-mpo.gc.ca](mailto:Caroline.Quinn@dfo-mpo.gc.ca)>; Seguin, Natalie <[Natalie.Seguin@dfo-mpo.gc.ca](mailto:Natalie.Seguin@dfo-mpo.gc.ca)>; Fagan, Ashley



<Ashley.Fagan@dfo-mpo.gc.ca>

**Subject:** For RDG approval

Hi Louise, [REDACTED]

This has been updated to reflect the events of last week. Can you send this up to Rebecca svp for formal PAC Region approval? Via Science and AMD there, but of course!  
(Marian has OK'd at this end.)

Also, I spoke to Arran this am about the notion in the plan to create a position/function for a person there whose job it is to deal with ENGOS/public/media on aquaculture (I call the person [REDACTED] of Aquaculture), and she was supportive.

If I could get this back by Wednesday COB, excellent.

Talk later...

Phil

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# **Communications Approach**

## **A Renewed Approach to Communicating Aquaculture**

### **Issue**

**DFO has recently made significant policy and funding announcements to demonstrate a renewed approach to aquaculture in Canada.**

**Together, the department's new approach--and the need to address the public's continuing concern about the industry's potential environmental impacts, particularly on wild salmon—represent an opportunity to renew and strengthen how DFO communicates about aquaculture—and in doing so, provide comfort to Canadians that aquaculture activities are being undertaken in a manner that is environmentally responsible and sustainable.**

**As an additional driver for this new approach, recommendations to improve communicating aquaculture figure prominently in the recent *Report of the Independent Expert Panel on Aquaculture Science* by the Office of the Chief Science Advisor of Canada, and are addressed here.**

### **Background/Context**

**In British Columbia--and to a smaller but growing extent in NL—a vocal ENGO community opposed to open pen aquaculture often leads the public and media conversation. The conversation is sometimes incorrect, emotional, and often centres on a widely-held suspicion that DFO promotes the aquaculture industry and is therefore conflicted in its regulatory/oversight role, to the detriment of wild salmon populations.**

**Overall, coverage of Canadian aquaculture since April 2018 to present has been neutral to critical in tone. DFO news releases and Ministerial events regarding new aquaculture and wild salmon protection funding/initiatives get factual but limited mention in the media, and are often overshadowed by negative social media chatter led by ENGOs and activists, (ie First Nations' protests, 'Bloodpipe' effluent video; PRV tech briefing held February 7/19), and negative opinion pieces in the print media.**

**What does GoC-led public opinion research say about Canadians attitudes toward aquaculture? A recent survey led by PCO found that:**

- **Canadians' familiarity with aquaculture and fish farming in Canada is fairly low. Overall, 24% of Canadians are paying attention to it, including 10% who are discussing it with others.**

- There is a significant divide across regions with residents from the east and west coasts (Atlantic provinces - in particular Newfoundland and Labrador, and British Columbia (45% each)) paying significantly more attention than residents of other regions. Older Canadians (55+) are also more likely than younger Canadians (18-34 years old) to pay attention to aquaculture.
- Canadians get their information about aquaculture and fish farming mostly in the news (TV, newspaper) and on the Internet. While older Canadians (55+) are significantly more likely to rely on traditional media, younger Canadians tend to rely more on the Internet. Perhaps not surprisingly, social media are not an important source of information about aquaculture in Canada.
- Residents from British Columbia are significantly more sensitive to the environmental risk of fish farming. Three in five (57%) think that the risk to the environment is high and only 15% think it is low.
- When asked about the credibility of fish farming information from various groups, Canadians consider scientists to be the most credible by a large margin.

While a more detailed Ekos survey will be available by the end of the fiscal year, the PCO survey points to areas where DFO Communications can deploy new efforts.

## **Objectives**

- Demonstrate to Canadians that the Government of Canada has a new approach that ensures aquaculture activities are undertaken in a manner that is environmentally sustainable.
- Increase awareness and improve public understanding and confidence in DFO regulatory activities.
- Emphasize DFO is not an industry promoter.
- Address the NGO community, particularly in BC and NL--as a key and distinct DFO audience that requires new, targeted efforts from the department.
- Showcase DFO's commitment to providing open and transparent scientific and management information about aquaculture to Canadians.
- Highlight the intention to build upon aquaculture regulations and activities in partnership with stakeholders, Indigenous groups and partners.
- Clearly and plainly explain how scientific evidence and the precautionary approach are applied when making aquaculture management decisions.

## **Audiences**

**Primary audience: 55 years+ Canadians in BC and Newfoundland who are following finfish aquaculture issues most closely via traditional media. (TV, Print, Radio, internet)**

**BC and Atlantic ENGOS**

**Secondary audiences:**

**Media, particularly in BC and NL, who regularly report on aquaculture issues**

**Academics**

**Indigenous Groups**

**Aquaculture Industry**

**General public (other than 55+ in BC and NL and Labrador)**

**Members of Parliament**

## **Strategic Considerations**

- **ENGOS lead the public discourse on aquaculture in BC, and are growing in influence in NL where aquaculture operations are proposed to expand. The ENGO community is a key and distinct DFO audience that requires more of our information, more often. To be effective, particularly in Pacific Region, additional resources may be required to begin and sustain this effort. It is not realistic to expect ENGOS' positions regarding open-pen aquaculture's impacts on wild salmon will change 180 degrees by the application of DFO communications efforts. However, the content and tone of the ENGO-led discourse (often given prominence by BC media) suggests strongly that there is co-ordinated work to be done by the Communications Directorate, Science Outreach and Aquaculture Science and Management sectors that can, at minimum, educate these groups about DFO's new approach to aquaculture that might better inform--and bring some accountability to--the ENGO-led discourse.**
- **55+ year olds in BC and NL are most engaged in the aquaculture conversation, and have the most concerns about aquaculture's impact on the environment. They get most of their information via print, radio, television, and the internet. Proactive media relations efforts should target this audience, and these traditional media, via an aggressive push for op-eds, letters to the editor, conducting editorial boards, and organizing ride-alongs to aquaculture sites, particularly for television journalists. DFO's Aquaculture web pages should be strengthened and the language made more accessible to the Canadian public.**
- **Exercise caution when using social media as a Communications tool; going out on aquaculture via social media frequently results in extreme responses.**

- Capitalize on the Minister's participation as the lead spokesperson for a new approach to aquaculture.
- The Report of the Independent Expert Panel on Aquaculture Science makes several recommendations to the department on how to improve communications to Canadians about DFO Aquaculture Science. The Tactics and Tools annex of this Comms plan addresses several of those recommendations.
- The headline from the Spring 2018 Commissioner of the Environment and Sustainable Development report was 'DFO did not adequately manage the risk associated with Salmon Aquaculture consistent with its mandate to protect wild fish.' The department agreed with the Commissioner's recommendations and is addressing her findings. Future announcements about new aquaculture policies and programs should include prominent messaging on how new aquaculture policies/programs/funding protect wild salmon.
- The government has made recent and significant policy and funding announcements toward modernizing aquaculture operations, i.e., Fisheries and Aquaculture Clean Technology Adoption Program project funding; Area-Based Aquaculture Management; a new Framework for Aquaculture Risk Management; the State of Salmon Aquaculture Technology Study; new Federal Aquaculture Legislation; \$100M toward the creation of the BC Salmon Restoration and Innovation Fund, and further \$5M funding for the Pacific Salmon Endowment Fund, among others. All of these above initiatives provide opportunities for announcements as key milestones are met, and programs and consultations (esp. a new Aquaculture Act) are launched.
- The Province of BC and First Nations recently announced consultations on the removal/relocation of Atlantic salmon farm pens in the Broughton Archipelago. DFO supports this initiative and has offered to work with the parties going forward. Depending on the nature of that DFO work, the department may consider raising awareness of it via Communications efforts, as it may have thematic links to the recently announced Area-Based Aquaculture Management initiative.
- 'Namgis First Nation and BC activist [REDACTED] launched separate applications in Federal Court challenging the department's policy of not requiring testing for PRV in smolts before authorizing transfers from hatcheries to aquaculture pens. A court decision rendered February 4/19 directed DFO to reconsider its PRV policy, and consult properly with 'Namgis FN. The department has until March 4 to consider whether to appeal, and until June 4 to reconsider its PRV policy.
- Use new Ekos Public Opinion survey results (expected mid-February 2019) to guide future communications activities.

## **Storyline**

- **The Government of Canada has a new approach to aquaculture that ensures activities are undertaken in a manner that is environmentally responsible, sustainable, and protects wild salmon.**
- **Together with Indigenous, environmental and industry partners, we will lead the way towards a more prosperous and sustainable aquaculture industry.**
- **We openly encourage innovation and discussion about new ways to improve aquaculture in Canada.**
- **We recognize the concerns some critics may have about the environmental impacts of aquaculture.**
- **Through research, DFO scientists help to better understand the impacts of aquaculture on our environment and improve our regulatory management of the industry.**
- **DFO commits to making its aquaculture science research, information on science-related activities and publications readily accessible to Canadians.**

## **Communications Activities and Tools (See Annex 1)**

## Opportunity/Activity

## Rationale

## Lead(s)

## Status

## Completion Date

### From Now until June 2019

Launch new Aquaculture 'Campaign' pages on DFO website to support Aquaculture Act consultations, management and science information

Provides portal through which public, media and stakeholders can read about our renewed approach to Aquaculture, engage in major Aquaculture Management Directorate initiatives such as consultations on a new Aquaculture Act, Technology Study, Area-Based Management, FACTAP projects, Framework for Aquaculture Risk Management

AMD/SRS/Strat Comms NHQ

Development pages have been drafted/designed. Looking for AMD signoff

March/ 2019

Continue Media Technical Briefings on Pathogen and Virus Risk Assessments for BC

Demonstrates DFO Aquaculture Science transparency. Risk Assessments (RAs) to be completed by September 2020 as per Cohen Commission/CESD Spring 2018.

Science ADM/NHQ Strat Comms

Next tech briefing Briefing on PRV held February 7, 2019, sixth in a series of ten. (TBC)

Ongoing to September 2020

Namgis decision response/appeal

Minister has until June 4/2109 to reconsider PRV policy as per court decision rendered February 4/19. Minister has until March 4 to decide whether to appeal Namgis decision.

NHQ and PAC Comms/Legal/AMD

Developing

Appeal decision Formatted: English (Canada) March 4; PRV Policy reconsider by June 4/2019 Ongoing

Proactive media pitches to TV, radio, print journalists on DFO aquaculture initiatives/science/operations esp. in BC and NL targeted at 55+ demographic.

Responds to PCO Public Opinion Survey findings re: PCO survey, targeting demographic most concerned/tuned into aquaculture issues. Builds on recent PAC Region success with media ride- along.

Regional Strat Comms in BC and NL.

Proactive media pitches to be developed and pitched to MinO via NHQ Strat Comms

Minister attends editorial board at the Vancouver Sun re: new approach to Aquaculture

A key Ministerial speaking opportunity to reach the 55+ audience in BC that relies on traditional media to get their information on aquaculture

PAC Region and NHQ Communications

Aim for March

TBC

Create a new function/position on a two-year assignment basis in Pacific Region to conduct

Recognizes that NGO and academic community is a distinct key audience for DFO; responds to Public Opinion

NHQ and PAC Region Aquaculture Science and

For consideration. If agreed,

TBD

regular and proactive DFO Aquaculture information sessions with the NGO community in BC, advocates, academics, the public and media. ( ) of Aquaculture)

Research that suggests the public places high credibility in information received from NGOs, academics, the media, and that some of this information is mischaracterized, inaccurate and/or out of date.

Aquaculture Management Program areas; NHQ and PAC Strategic Comms;

move to implement asap.

Twitter chat or Facebook Live event with DFO Aquaculture Scientists

Allows public participants' transparent access to interact directly with DFO Scientists

Science ADM/Science Outreach

Developing

Science Odyssey Week May 2019

Formatted: English (Canada)

Aquaculture Video Production

'From Innovation to Validation' video features aquaculture management and two research/technology approaches being tested to assist in monitoring impact from aquaculture.

NHQ Science Outreach

In editing

TBC. Will be posted on DFO Aquaculture web pages, and featured at (ASEC/PSEC, one of Ingenium's museums (other venues?))

## Announcements

Announce New Science Advisor

Responds to Chief Science Advisor direction.

ADM Science/NHQ Strat Comms

TBD

TBD

Announce External Advisory Panel on Aquaculture

Responds to Aquaculture Expert Panel report recommendation

Science ADM/NHQ Strat Comms

Panel to be designated by new Science Advisor

TBD

Next FACTAP funding announcement

Funding for environmental innovations/improvements/efficiencies related to aquaculture operations. Positions department as leader in supporting green technologies for aquaculture operations.

AMD/NHQ Strat Comms/NL Comms

Mid-February 2019 announcement/Ministerial event

St. John's, NL, TBC

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Promote progress/milestones regarding Area-Based Aquaculture Management Initiative (ABAM) with program partners.	Supports Dec 10/19 Ministerial announcement to enhance collaboration among federal, provincial and Indigenous partners when planning, monitoring and ongoing management of aquaculture activities. Proactive Communications can occur once pilot project area is selected, expected to be North Vancouver Island area.	NHQ AMD/PAC AMD/PAC Comms	Pending ABAM site selection	Discussions with BC, Indigenous Peoples and communities
Promote progress/milestones in Framework for Aquaculture Risk Management Promote State of Salmon Aquaculture Technology Study results.	Proactive Communications to further publicize a new framework for risk management in aquaculture. Positions department as an agent of innovation, modernizing environmentally sustainable aquaculture operations. Study led by DFO with Sustainable Development Technology Canada, BC government with Indigenous partners.	NHQ <u>Comms/AMD/NHQ SRS</u> NHQ AMD/NHQ Strat Comms/Sustainable Development Technology Canada	TBC  Study expected to be completed by Spring 2019.  News Release announcing study completed will point public/stakeh olders to study published on DFO web pages.	TBC  Final report released Summer 2019
Publicize a completed New Framework for Aquaculture Risk Management to clearly explain how we make fishery decisions while incorporating the precautionary approach.	Responds to recommendations from the Commissioner for the Environment and Sustainable Development, industry, and from Canadians who have asked for more transparency on how aquaculture decisions are made.	NHQ AMD/NHQ Strat Comms	TBD	TBD
Canadian Aquaculture Research and Development Review 2019	Biennial review is a compendium of aquaculture research and development projects from across Canada to be posted on DFO website. Positions department as agent of innovation in Aquaculture.	SRS/Science Outreach/Strat Comms.	Editing and layout underway	TBC

## Longer Term Initiatives

Revamp DFO Science website	Responds to Aqua Expert Panel report recommendation; will provide better public access to DFO information on Aquaculture science, research, Science programs, publications	NHQ Web Services/Strat Comms	Project proposal in development	Winter 2020
Develop as a priority, aquaculture public education programming at DFO's two Science Enterprise Centres (PSEC/ASEC). These venues can regularly feature public exhibits/seminars/presentations about what DFO Aquaculture Science and Management programs are doing to assure aquaculture is environmentally sustainable.	Responds to Aquaculture Expert Panel report recommendation to achieve dual goal of informing citizens while contributing to broad science education objectives.	NHQ Science Outreach; Office of Partnership and Collaboration; PAC Strat Comms, Gulf Strat Comms, PAC Science Program, Gulf Science Program	ASEC currently supporting phase II of a project on oyster aquaculture. PSEC program to be developed.	Ongoing
Canadian Geographic (CanGeo) Special Issue on International Year of the Salmon	Shows transparency and willingness to address issues and perceptions related to salmon and aquaculture. Allows DFO to broaden our reach (for IYS and around aquaculture) to more CanGeo's iconic magazine has a reach of 3.1 Canadians per month. Web resources have a reach of 1.1 million Canadians per month, and a 21,000 teacher network.	Science Outreach; Aquaculture Science Program NHQ; Strategic Comms, Science ADM; Regional Science Participation	Early discussion with Can Geo - DFO considering the potential. Idea to be raised at IYS Communications working group.	Potential Fall issue – 2019 (IYS signature year)
Pursue proposal to partner with the Canada Agriculture and Food Museum within context of food security. 2022 launch of long term exhibit in CAFM Learning Centre, travelling exhibit for pan-Canadian tour; national education modules, national advisory council and Indigenous Foodways Partners	Responds to Aquaculture Expert Panel report recommendation to broaden public outreach and form strategic alliances to communicate about aquaculture information in publicly accessible places.	Science Outreach; Office of Partnership and Collaboration (Part of a larger proposal to partner with <i>Ingenium</i> —Canada Science and Tech Museum and Technology Museum)	DFO considering proposal (partnership would require multi-year funding commitment).	Exhibition launch 2022-23

<b>Public in-person speaker series participation - alternate venues</b>	Allows public participants' transparent access to interact directly with DFO Scientists	Science ADM/Science Outreach	TBD – could include a “Curiosity Stage” event at Museum of S&T and/or museum of nature speaker.	TBD
<b>2022 is UN-declared International Year of Artisanal Fisheries and Aquaculture</b>	Opportunity to position DFO as world leader in aquaculture Science and Management	NHQ and Regional Strat Comms/Science Outreach	TBD	2022

## Miller-Saunders, Kristi

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**From:** Higgins, Mark  
**Sent:** February-22-19 4:29 PM  
**To:** McLeod, Patricia; Kreiberg, Henrik  
**Cc:** Miller-Saunders, Kristi; MacDougall, Lesley; Lowe, Carmel  
**Subject:** RE: Stressors facing Chinook Salmon  
**Attachments:** Southern BC Chinook Health Higgins.ppt

As far as I can tell, this is a pretty broad request to summarize all the stressors facing Chinook salmon. Gayle Brown along with others organized a 2 day work shop back in 2013 to look at stressors facing chinook salmon (freshwater and salt water). A final report was to be drawn up by [REDACTED] (ESSA Technologies in Vancouver) to provide a summary of the workshop and stressors facing chinook salmon. I don't have that report (I found a link to it, but it did not work), so you may want to talk to Gayle Brown.

I have included the presentation that we put together for the workshop summarizing pathogens that are potential stressors to Chinook salmon, BKD topping the list as Chinook salmon are very susceptible can often having underlying infections. However, disease mitigation at both Federal Hatcheries and marine finfish farms help to reduce pathogen load.

Mark.

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**From:** McLeod, Patricia <Patricia.McLeod@dfo-mpo.gc.ca>  
**Sent:** February-22-19 3:41 PM  
**To:** Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Kreiberg, Henrik <Henrik.Kreiberg@dfo-mpo.gc.ca>  
**Cc:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Subject:** FW: Stressors facing Chinook Salmon  
**Importance:** High

Hi Mark and Henrik,

See Kristi's note below.

Kristi doesn't not specify a deadline time on this but it would appear that it is due as soon as possible on Monday to allow Kristi to put it together.

Carmel's note advises "the request is for a Plain Language Summary" .... So I have attached one here for your use – not sure that you are able to complete all the boxes but it might help as a guide.

Please send your responses directly to Kristi. Kristi advises she will "roll-up" on Monday. Make sure you cc Lesley and also me.

Many thanks,

s.19(1)

Trish  
7169

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**From:** Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>  
**Sent:** Friday, February 22, 2019 3:23 PM  
**To:** McLeod, Patricia <[Patricia.McLeod@dfo-mpo.gc.ca](mailto:Patricia.McLeod@dfo-mpo.gc.ca)>  
**Subject:** FW: Chinook

Can you please fan this out to other section heads in ADGT for response? I will compile what I can Monday.

Thanks,  
Kristi

---

**From:** Lowe, Carmel  
**Sent:** February 22, 2019 3:13 PM  
**To:** Miller-Saunders, Kristi  
**Cc:** MacDougall, Lesley  
**Subject:** Chinook

Hi Kristi

We have received a request from the Minister's office to provide information on the stressors facing Chinook salmon. Due date is COB Wednesday next, Feb 27<sup>th</sup>. In Lesley's absence can you provide a few bullets on this drawing from the work being undertaken in ADGT please? The request is for a plain language summary. Folks in Eddy's and John's Division's will also be feeding into this.... So we will need some time to stitch the contributions together.

*Carmel*

Carmel Lowe, Ph.D.  
Regional Director Science | Directrice régionale des sciences  
Fisheries and Oceans Canada | Pêches et Océans Canada  
Pacific Biological Station | Station biologique du Pacifique  
3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7  
[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)  
Telephone | Téléphone 250-756-7177  
Facsimile | Télécopieur 250-729-8360  
Government of Canada | Gouvernement du Canada

# **Has Mortality from pathogens (including from hatcheries and aquaculture) during either freshwater or ocean phase contributed to changes in productivity or abundance of Southern Chinook Stocks?**

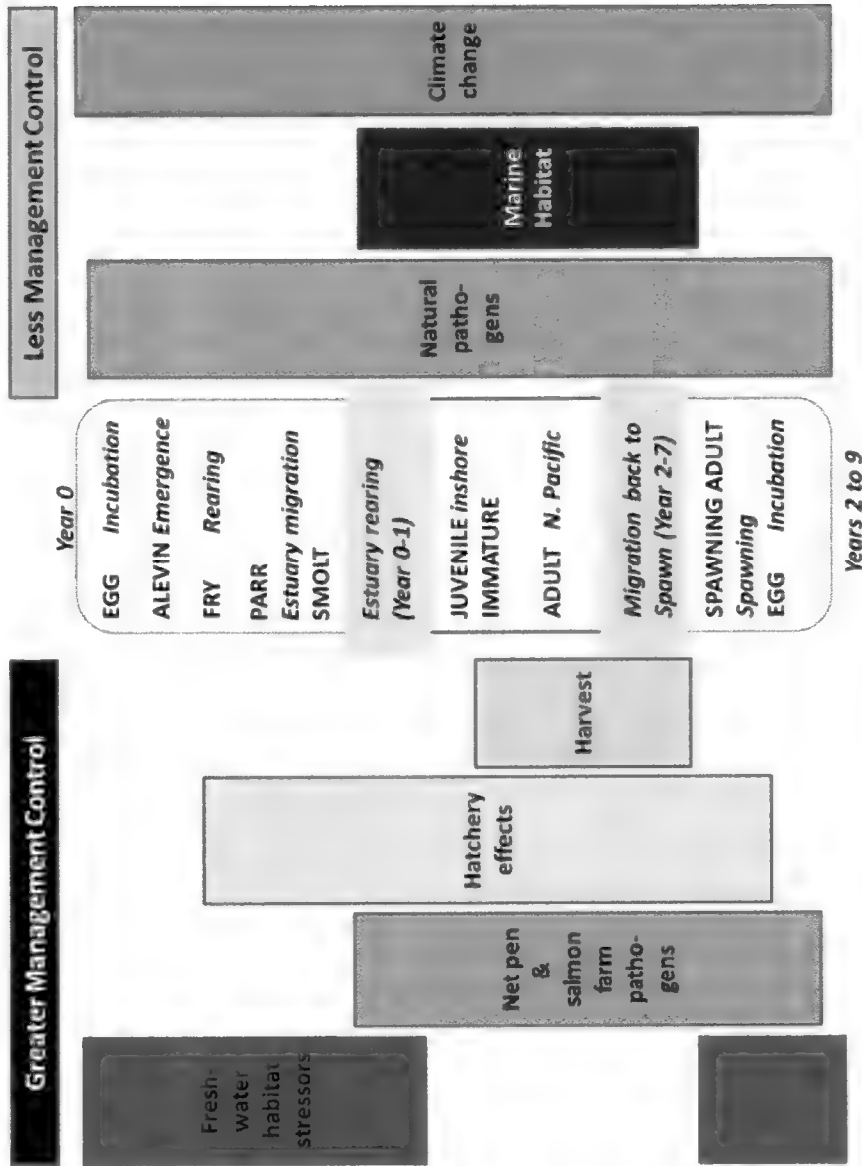
Contributors: Mark Higgins, Kyle Garver, Simon Jones, Chris  
MacWilliams, Stewart Johnson and Kristi Miller

Fisheries and Oceans Canada  
Pacific Biological Station  
Nanaimo, BC





# Southern BC Chinook Conceptual Model

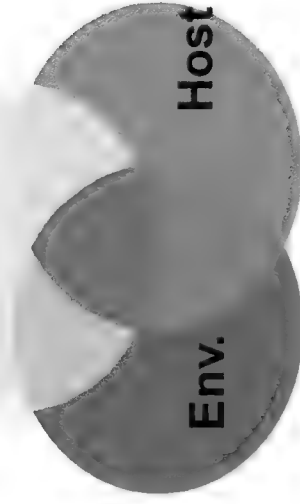




## Pathogen vs. Disease

- Pathogens are a natural component of all ecosystems
- Pathogens co-evolve with their host
- Presence of pathogen  $\neq$  disease
- Disease determinants are multifactorial

**pathogen**



■ Disease occurrence

• Features of the host, the pathogen, and the environment determine the nature of the resulting disease

• Disease severity may increase when conditions change (upset the normal balance)



## Challenges to quantifying disease impact in populations

- Fish mortality due to disease can go unnoticed or underestimated due to the difficulties in finding and recovering carcasses
- Difficult to quantify indirect disease impacts on host populations (i.e. infectious disease could cause increased susceptibility to predation)
- Often little is known about the relationship between infection and disease.
- Occasionally the balance can be upset due to environmental extremes (i.e. warm temperatures, low dissolved oxygen) and disease/mortalities will result

Further complicated by:

- Limited knowledge of host-pathogen interactions
- Large number of stocks
- Different timing of seawater entry
- Many sources of origin



## Endemic Pathogen vs. Exotic Pathogen

### Endemic:

1. Present in a community at all times
2. Disease of low morbidity that is constantly present in an animal population, but clinically recognizable in only a few
3. Occurs with predictable regularity
  - Balance exists between fish immune response and pathogen
  - Occasionally the balance can be upset due to environmental extremes (i.e. warm temperatures, low dissolved oxygen) and disease/mortalities will result
  - But generally speaking – co-exist without obvious harm to wild populations

### Exotic:

1. A disease that does not occur in the geographic region
2. Infectious pathogens that may be introduced
  - Not in balance
  - No co-evolved immunity (i.e. *IHN* - in *Pacific salmon* vs. *Atlantic salmon*)
  - Recognize there are other sources of exotic pathogens than aquaculture or enhancement hatcheries (ballast water, seafood imports & processing, ornamental fish, etc.)





## Common pathogens of Chinook Salmon

- **Bacteria**
  - *Renibacterium salmoninarum* (BKD)
  - *Aeromonas salmonicida* (Furunculosis)
  - *Yersinia ruckeri* (ERM)
  - *Flavobacterium branchiophila* (BGD)
  - *Vibrio* Spp. (Vibriosis)
  - *Pseudomonas* Spp.
  - *Piscirickettsia salmonis*
  - *Flexibacter* (Columnaris disease)
- **Viruses**
  - Infectious hematopoietic necrosis virus (IHNV)
  - Viral Erythrocytic Necrosis virus (VEN)
  - Erythrocytic Inclusion Body Syndrome (EIBS)
  - Viral Hemorrhagic Septicemia virus (VHSv)
  - Pacific Salmon Paramyxovirus (PSPv)
  - Piscine Reovirus (PRV)
  - Salmonid herpesvirus
- **Parasites**
  - *Parvicapsula kabatai*
  - *Ichthyophthirius multifiliis*
  - *Loma salmonae*
  - *Eubothrium salvelini*
  - *Myxobolus cerebralis*
  - *Tetracapsula bryosalmonae* (PKX)
  - *Creatomyxa shasta*
  - *Lepeophtheirus salmonis*
  - *Caligus clemensi*
  - *Cryptobia salmositica*
  - *Nucleospora salmonis*
  - **Fungus**
    - *Ichthyophonus hoferi*
    - *Phoma herbarum*
    - *Sphaerothecum destruens*



## Bacterial Kidney Disease (BKD)

- Is found in all Chinook stocks from Alaska to Oregon
- Losses reported at most hatcheries each year due to BKD outbreaks (continual need to manage around this bacteria in Chinook salmon)
- BKD screening program for both fall and Spring release chinook from hatcheries (major facilities)
- 5 years of data from pre-release screening of hatchery stocks 117/827 (14%) of fish tested positive. Of these 4.7% considered high positive (possible destruction)
- Returning adult BKD screening program (Chris?)
- Less information on "wild" Chinook as we do not screen them (gap)
- Net-pen release information?
- Presume that returning adult prevalence is similar to hatchery numbers? However, not removed from population.





## Vibriosis and Furunculosis

- Both pathogens show up in Fish Health Database in all CU's. Hatchery practices are common to treat or vaccinate prior to release
- Cause issues in seawater out planting in net-pens for hatcheries
- Most stocks undergo dip vaccine prior to out planting. Size at release for SEP stocks only affords temporary immunity (6 months)
- Increasing mortality in sea-pen operations is cue to release (no health testing information on these releases) stock – safety concerns of personnel

## Piscirickettsia salmonis

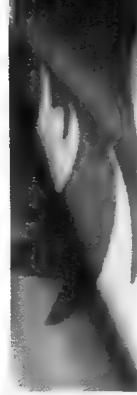
- Usually associated with other pathogens in net-pen reared chinook salmon.
- Has caused large mortality in net-pens in Chile - mostly in Coho salmon
- Still found in BC farming operations. Causes issues in confined populations like net-pens
- Not much data available for wild chinook





## Parasites

- Varying degrees of impact from parasites depending on life stage of the salmon
- Parasite load is often determined by environmental factors (water quality, temperature, dissolved oxygen, flow)
- *Tetracapsuloides bryosalmonae* (PKX) affects fry stage in fresh water (localized to some Vancouver Island water systems)
- *Ichthyophtherius multifiliis* (Ich) affects returning adults in northern Rivers and some Fraser river systems
- *Loma salmonae* can infect fish in both Fresh and Seawater environments. Direct transmission. Has been found to cause mortality in farmed Chinook salmon and speculated that wild Chinook act as reservoir for farmed populations (Kent and Poppe 1998)
- Studies from Washington and Oregon on Trematode (*Nanophyetus salmincola*) infections that indicate population level effects on out-migrating Coho and Chinook smolts (usually in conjunction with BKD?)



## Cultured fish vs. wild fish

- Live outside the 'balanced' relationship in the wild
- If disease starts, easier spread between individual fish due to close confines
- Perceived as having more disease than wild stocks – but biased, as continual monitoring for pathogens and disease occurs
- Re: risk of pathogen spread from hatcheries to wild fish - balance between endemic pathogens and wild fish is already established, periodically adding more is unlikely to cause significant harm





## Federal Hatchery Program

- Use local returning broodstock and release progeny to native watersheds
- Practise good husbandry and optimized nutrition
- Biosecurity measures:
  - Egg disinfection; brood selection and screening; daily monitoring for early disease detection; dedicated no-cost DFO diagnostic lab and vet; consultation on disease prevention and treatment; stock destruction as last resort
- No information on non-enhanced stocks as there is no sampling. Cannot tell difference between hatchery and wild as not all fish marked



## Enhancement hatchery philosophy

- Raise fish, not food
- Conserve and supplement wild runs and enhance fisheries opportunities
- Use surface water to allow imprinting and to encourage development of natural immunity to native pathogens
- Goal is to produce and release healthy fish, but without a competitive advantage over wild stocks

## Hatchery disease information

- BKD screening data for both juveniles and returning adults
- Incidence of disease in hatchery setting
- Use of antibiotics for hatchery fish (management strategies)
- Sea-pen projects
- Vaccine dips
- Most common pathogens on WCVI (364 entries from FH Database)  
Furunculosis, BKD, Yersinia ruckeri (ERM)
-

## Aquaculture Effects

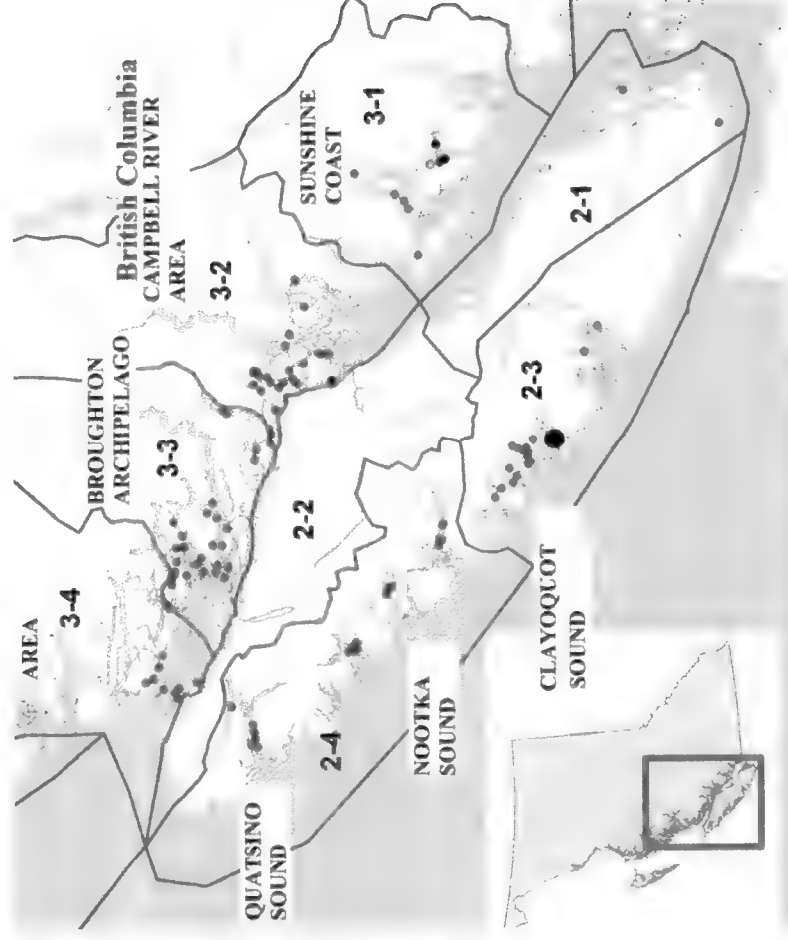
- Small number of chinook salmon farms
- Have same basic issues with pathogen types as hatcheries (i.e. BKD)
- Use management strategies to minimise disease events (low stocking densities, dip vaccinations, good management practices)
- Atlantic salmon farms more numerous, however, less overlap of pathogens between chinook and Atlantic salmon to cause disease
- Management strategies in place for vaccination programs (IHN, BKD, Furunculosis, Vibriosis) and good management practices



## Regulations minimizing risk of importing exotic pathogens

- All imports must meet conditions of fish health Protection Regulations
- British Columbia has egg import only policies for both Pacific and Atlantic salmon
- Strict quarantine and isolation upon entry to BC. No seawater entry for approximately one year
- Regular health testing throughout this period of time
- Authorities now taken over through CFIA

## Current Aquaculture facilities along the southern range of BC



## Chinook salmon pathogen studies in the U.S.

- Chinook and Coho salmon studies from the wild (Washington, Oregon) Jacobson et al. 2008, Arkoosh et al. 2004
- Evidence of reduced swimming ability leading to increased predation in trout ( *Nanophyetus salmincola* ) infected chinook and coho
- PRV detections in Washington and Oregon? Ask Chris for update from last meeting
- Ichthyophonus in Chinook and herring in Alaska and Yukon River
- Ceratomyxa shasta studies in Oregon and Washington State

## Gaps in knowledge

- Insufficient resources to monitor out-migrating stocks of non-hatchery Chinook in Rivers where major facilities located
- Parasite and pathogen information incomplete in out-migrating and returning Chinook



## Research Needs

- Disease profile of out migrating chinook from major rivers and stocks of concern
- Match up stocks of concern with pathogen profile
- Health data on sea-pen release chinook



## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** February-25-19 12:58 PM  
**To:** Withler, Ruth  
**Subject:** FW: stressors  
**Attachments:** Stressors facing Chinook Salmon.docx

Can you add a bit of very high level detail to the first line in this document being prepared for the minister? I need ASAP as it needs to go out today.

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**From:** Miller-Saunders, Kristi  
**Sent:** February 25, 2019 12:45 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** stressors

## Stressors facing Chinook Salmon

- Genetic introgression between hatchery and wild fish (RUTH)
- Infectious Disease: Chinook salmon carry the highest diversity of infectious agents, including viruses, bacteria and parasites, of all species of salmon tested in BC. Many are naturally occurring in freshwater habitats, others emanate from marine sources. Increased environmental stress can trigger epizootic outbreaks even of agents that are normally tolerated. Increased temperature can increase pathogenicity through effects on host immunity and pathogen replication. ***While naturally occurring endemic pathogens infecting wild salmon are difficult to control, our strongest potential for mitigation is through minimizing risks of transmission from cultured (enhancement and aquaculture) fish. This can include:***

- 1) reducing vertical transmission through broodstock testing in hatcheries,***
  - 2) testing and treatment (when available) of infected cultured fish prior to release,***
  - 3) limiting open-net aquaculture on Chinook salmon in regions where natural populations of Chinook salmon co-occur,***
  - 4) implementation of semi-closed or closed containment systems with organic removal to on-land sites,***
  - 5) refining minimum fallowing times using empirical scientific evidence (e.g. environmental DNA monitoring of infectious agents) to prevent reinfection,***
  - 6) re-visiting siting criteria to minimize transmission potential between farms (reduce farm densities) or fallowing areas of high density synchronously, and***
  - 7) regulating fish processing plants and well boats with high densities of fish destined for harvest to require on land disposal of organic waste.***
- There are a number of recently discovered viruses in Chinook salmon that increase during smoltification and upon entry into saltwater. There needs to be focused effort to understand the role of these viruses in early marine survival. One is largely emanating from SEP hatcheries, a relatively easy control point if proven pathogenic.
  - There has been an increase in gill diseases in farmed salmon in recent years; some may be due to cnidarians (jellyfish), and others to parasites and bacteria infecting gill tissue. Wild salmon share the same mixture of gill pathogens as do farmed fish, including virtually all agents associated with gill diseases in Norway.
  - Piscine Orthoreovirus (PRV) infection in farmed Chinook salmon is associated with the development of the disease Jaundice/Anemia, which has caused chronic low-level mortality over winter on farms for 15 years. Wild Chinook salmon overwintering in Quatsino sound have now been shown to carry the same pattern of lesions associated with high PRV loads as observed on farms during the winter period. PRV challenge studies have also reproduced many of the early gross and microscopic lesions associated with this disease.
  - Aquaculture impacts in terms of pathogen transmission may be strongest felt on the west coast of Vancouver island, where juvenile Chinook salmon spend several months

rearing in the bays and inlets also occupied by farms. These inlets also serve as overwintering habitats for a mixture of Chinook salmon stocks. ***Before there is a move to increase farms along the north coast, it will be important to better understand the risks of farm to wild transmission of infective agents in similar environments on the west coast of Vancouver Island, which are quite divergent from the Discovery Island situation.***

- Exposure to harmful algal blooms is expected to continue to rise under conditions of increased ocean acidification; it is not clear whether salmon farms may also impact bloom production. Blooms can also impact oxygen levels, and cause hypoxia in salmon, a major threat to farmed salmon, and a threat that is not yet well characterized for migratory salmon. ***If farms do impact bloom cycles through high organic waste, implementation of semi-closed containment systems that include on shore disposal of organic waste could reduce harmful algal bloom incidence and risk.***
- If SLICE resistance on farms continues to develop and expand along the coast, and other mitigation measures to control sea lice are not effective, increased sea lice levels on juvenile salmon pose a threat to juvenile Chinook salmon, especially in inlets shared by farms. Increased handling of aquaculture fish to control sea lice could also increase their susceptibility to infectious disease, something that should be carefully monitored. ***Implementation of semi-closed containment systems could significantly reduce sea lice threats and handling, thereby minimizing risks to migratory Chinook salmon.***
- Release of hatchery fish outside of the smolt window (before they are physiologically ready for the transition to saltwater), can affect their ability to adapt and feed, and increases susceptibility to disease and other stressors. This is particularly problematic for release of post-smolts (fish that have started de-smolting). ***Genomic tools have been developed to precisely stage smolt window and if implemented, could increase early marine survival of releases. Before a significant expansion of hatchery Chinook salmon release is considered, all available means to track and increase the health and condition, and smolt readiness, of hatchery fish should be implemented.***
- Seal predation of salmon leaving estuaries can be high. This may impact hatchery fish more than wild fish due to the release of large pulses of fish. ***There is a possibility to reduce impact by slow release of hatchery fish and removal of haul-outs (e.g. log booms) for seals in estuaries.***
- Inadequate food resources in early marine environments in years of higher temperature are a risk. ***Optimising the condition of hatchery fish, which are most vulnerable to variations in food abundance, could increase survival.***
- High temperatures in river migration of return migrants are a threat, with high mortality demonstrated during extended periods >18°C. ***Limitation of additional stress, such as fisheries, during high river temperatures could reduce mortality.***
- Fish that escape but are wounded by gill net fisheries in river experience significant mortality and enhanced disease development if river temperatures are >16°C. ***Reduction in fisheries during high river temperatures could alleviate some losses.***

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** March-04-19 12:32 PM  
**To:** Waddington, Zac  
**Cc:** McConnachie, Sarah  
**Subject:** RE: Audit 2 paper

Hi Zac,

Thanks for getting hold of me on this and providing an overview of what you see is an important use of the audit data which my lab is intimately involved in as it was part of the SSHI. Hence, yes, I do see a role in the development in this paper; it is not "AVC" data, but rather they are part of the SSHI and working with the data we and the AMD have provided to them. We have, for over three years now, held weekly teleconference calls with the AVC on various papers being led by them with data provided by my lab, and I would expect if we are to pursue a second audit paper, which I support, that we would continue along these lines but link in those in the AMD that would like to be involved. I also agree that these three diseases are the most readily recognized and diagnosed in the farmed audits, although mouth rot is not diagnosed in the Pacific audits per se. As I have not seen Ian Keith's extra workup that was provided only to the AVC, I cannot comment on that.

I do not think you are going to find consistent patterns within the group of fish that do not classify neatly into one of these three endemic diseases, as these fish are not necessarily "non-diseased" as you classify them, they simply do not have one of your three listed diseases. This is certainly an area we are delving into with the network analysis. However, from what I read below you are looking to determine if other infectious agents may be over represented in the "disease" group, correct? The answer to that question is yes, in two cases. Below are the three clusters with these three diseases that we have resolved in network analysis of Atlantic salmon (on an individual rather than farm-basis):

### Community3

Histopathology (common): Tenacibaculum

Histopathology (uncommon): -

Clinical (common): necropsy.scores\_mouthrot, necropsy.scores\_brain.hemorrhage

Clinical (uncommon): -

Diagnostic (common): Ulcerative Stomatitis

Diagnostic (uncommon): Systemic\_dz, Meningitis\_Encephalitis, BKD, P\_Salmonis

Gene Biomarkers (common): IL.17D\_onmy, C7, hep\_onmy, TF\_onmy

Gene Biomarkers (uncommon): SRK2\_MGB3, SAC\_MGB2, UBL1\_MGL\_2, XAF1\_MGL\_1, GIG2.1\_MGB3, RSAD\_MGB2, IFI44A\_MGL\_2, MHCI.sasa1, MHCI\_sasa1, UBA\_MGL\_CA050178\_1, RTP3\_MGL\_1, BAF\_MGL\_4, CA054694\_MGL\_1, CA038063\_MGL\_1, IgMs\_onmy

Microbes (common): te\_mar

Microbes (uncommon): ascv, prv, ctv

### Community5

Histopathology (common): kid\_itis, liver\_itis, spleen\_wpulpitis, BKD, heart\_myocarditis, heart\_periocarditis, gill\_itis, cns\_itis

Histopathology (uncommon): -

Clinical (common): necropsy.scores\_visceral.white.foci, White\_Foci, Swollen\_Kidney, BKD, Pseudocapsule

Clinical (uncommon): necropsy.scores\_food.in.gut

Diagnostic (common): BKD

Diagnostic (uncommon): Ulcerative Stomatitis, Meningitis\_Encephalitis

Gene Biomarkers (common): CA038063\_MGL\_1, SAA\_onmy, BAF\_MGL\_4, UBL1\_MGL\_2, PLAUR\_MGL\_3, SRK2\_MGB3, MX\_ONTS, GAL3\_MGL\_2, PSMB9A\_MGL\_2, E3RNF213\_2, XAF1\_MGL\_1

Gene Biomarkers (uncommon): IL.17D\_onmy, NKA\_b1\_sasa, JUN

Microbes (common): re\_sal, ctv

Microbes (uncommon): te\_mar

Community7

Histopathology (common): Rickettsia, cns\_itis, kid\_osis

Histopathology (uncommon): -

Clinical (common): Fecal\_Cast

Clinical (uncommon): necropsy.scores\_food.in.gut

Diagnostic (common): P\_Salmonis, Meningitis\_Encephalitis, Systemic\_dz

Diagnostic (uncommon): Ulcerative\_Stomatitis

Gene Biomarkers (common): MMP13\_sasa, IL1B\_sa.om

Gene Biomarkers (uncommon): -

Microbes (common): pisc\_sal, sch, ctv, pa\_ther, prv

Microbes (uncommon): -

Community8

As you can see, both Rickettsiosis and BKD show both an over-representation of the agent causing these diseases, as well as other agents (CTV being common to both). In some cases of BKD diagnosis, they only have CTV, which is something that we have been looking at closely. Fish with Mouth Rot, on the other hand, tend to not have co-infecting agents. This may be partly explained by the very early incidence of this disease post seawater entry. But I believe you will find that other infectious diseases often develop after a bout of mouth rot.

I am not at all opposed to tackling these questions at different angles, and have thought that a focus on known endemic diseases is warranted. It will be important to also understand how often the agents causing these diseases are present on farms with no evidence of disease, whether there are thresholds of these agents associated with disease development, and how often these diseases have been diagnosed in the absence of the agents causing them (perhaps due to treatment, or something else).

So yes, please keep me in the loop on this one. I assume that Krishna would be leading? Is Raph continuing to stay involved? Or has it reverted to Ian?

Thanks,  
Kristi

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**From:** Waddington, Zac  
**Sent:** March 4, 2019 10:43 AM  
**To:** Miller-Saunders, Kristi  
**Cc:** McConnachie, Sarah  
**Subject:** Audit 2 paper

Hello,

We've been chatting with the epi folks from AVC about trying to get this second endemics paper published before some SSHI funding expires in June. I think this has been referred to as the "audit 2" paper in the past?? We've gone back and forth as to a good question which we think the data has the power to answer, and doesn't duplicate work your lab is doing. What we've landed on is looking at the infectious agent distribution in diseased and non-diseased fish as diagnosed by Ian Keith. This is the data that he was sensitive about sharing since it is very subjective, but he is comfortable with this approach. The diseases with enough diagnoses to provide power would be mouthrot, BKD and SRS. The hope would be that we could potentially get to the role (or lack thereof) of some other infectious agents which may act as a potentiating factors in manifesting clinical disease in a given fish. We would also look at stratifying the analysis by whether or not the farm had a farm-level disease diagnosis made at the time of audit sampling. This could help speak to the role that various co-infecting agents may play in facilitating an epidemic disease manifestation on farm, as opposed to an endemic disease manifestation.

I am understand that your lab is extremely busy with your current work, and this work wouldn't require any resources or support from your lab. That said, we would welcome your input to whatever degree you wish to be involved.

Let me know if you'd like to have a call or meeting to discuss this further.

Dr. Zac Waddington DVM, B.Env.Sc.(Hons)  
Lead Veterinarian - Pacific Region  
Fisheries and Oceans Canada | Pêches et Océans Canada  
Aquaculture Environmental Operations - Fish Health  
Courtenay, British Columbia  
Telephone | Téléphone: 250-703-0902  
Fax | Télécopieur: 250-703-0921  
[Zac.Waddington@dfo-mpo.gc.ca](mailto:Zac.Waddington@dfo-mpo.gc.ca)

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## Miller-Saunders, Kristi

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**From:** Parsons, Jay  
**Sent:** May-13-19 12:51 PM  
**To:** Miller-Saunders, Kristi; Waddington, Zac  
**Cc:** Olivier, Gilles; Mimeault, Caroline; Burgetz, Ingrid; Weber, Lily  
**Subject:** RE: PRV characterization paper for your official review

Kristi, Zac:

I am checking on the status of this request. Can you please let me know if you are able to respond today?

Thanks, Jay

**From:** Parsons, Jay  
**Sent:** Friday, May 03, 2019 3:35 PM  
**To:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Waddington, Zac <Zac.Waddington@dfo-mpo.gc.ca>  
**Cc:** Craig Stephen <cstephen@cwahc-rsfc.ca>; Olivier, Gilles <Gilles.Olivier@dfo-mpo.gc.ca>; Mimeault, Caroline <Caroline.Mimeault@dfo-mpo.gc.ca>; Burgetz, Ingrid <Ingrid.Burgetz@dfo-mpo.gc.ca>; Weber, Lily <Lily.Weber@dfo-mpo.gc.ca>  
**Subject:** PRV characterization paper for your official review

### On the behalf of the PRV CSAS Co-chairs (Craig and Gilles)

Dear Kristi and Zac,

We are contacting you about the DFO CSAS peer-review process for the PRV risk characterization paper that took place in January 2019. As the official reviewers identified at the CSAS meeting please review to determine if the requested changes from the meeting have been incorporated appropriately. Please send your review by **Thursday May 9<sup>th</sup>, 2019**. Your roles as official reviewers are vital and greatly appreciated.

See the attached revised version of the PRV characterization paper including the incorporated changes requested at the CSAS peer-review meeting. A table of requested changes and how they were addressed has also been included for your reference.

Please recall we are not seeking a full re-review of the paper but rather a verification that the requested changes were incorporated and the responses are appropriate for the requested change.

Thank you,

Jay

**Jay Parsons, PhD**  
**Director**  
Aquaculture, Biotechnology and Aquatic Animal Health Sciences Branch  
Fisheries and Oceans Canada / Government of Canada  
200 Kent Street, Stn 12E239 Ottawa, ON Canada K1A 0E6  
Jay.Parsons@dfo-mpo.gc.ca/ Tel. 613-990-0278



**Directeur**

Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques  
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du Canada

Canada

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## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** March-14-19 1:57 PM  
**To:** Polinski, Mark  
**Cc:** [REDACTED] Miller-Saunders, Kristi; [REDACTED] DiCicco, Emiliano  
**Subject:** Two Question on Paper on PRV in Nature  
**Attachments:** 2017-0400HSMIAdviceMorton-Interim.pdf; ATIP A2016-203 HSMI recognized.pdf

Dear Dr. Mark Polinski:

Congratulations on your publication in the high impact journal Nature.

I am writing for clarification on your statement that HSMI has been diagnosed in BC salmon in absence of PRV.

In your section titled **Preface concerning the diagnosis of HSMI**; you rely on two papers in making the statement that HSMI has been reported in farm salmon in absence of PRV: “... *if HSMI diagnosis is based solely on histopathological heart lesions which can occur in the absence of PRV*...” The papers you appear to cite are Marty et al (2015) and Di Cicco et al (2018).

This is an important conclusion, an outlier to other literature on PRV in Atlantic salmon, and it is a “preface” to the work you report on in your paper.

However, Di Cicco et al (2018) say that they suspect that the one fish reported the farm audit data with heart lesions in absence of PRV “*was not an HSMI fish*”.

Similarly, Marty et al (2015), state: “*None of the fish in our study had microscopic lesions diagnostic for HSMI*.” They noted heart damage, but suggest it was due to *Loma salmonae* infection, and did not view it as HSMI.

Neither of these papers support the conclusion that HSMI occurs in absence of PRV.

Similarly in 2016, yourself and your co-authors, reported that western North America is “*a region now considered endemic for PRV but without manifestation of HSMI*” (Garver et al 2016). This is a sweeping statement that includes all the farm salmon health audits, in all years and the private reports by Dr. Marty’s lab to industry. This suggest that prior to 2016 HSMI was never seen in BC.

However, four months *after* Garver et al (2016) stated that HSMI does not manifest in BC, Dr Marty appears to reverse his position in an internal email that he did find HSMI-like lesions in BC farm salmon beginning in at least 2008 (attached).

When I asked Dr. Marty to describe the difference between HSMI and HSMI-like he said there was “no difference” (attached). Excuse me if I have this wrong, but this suggests that when Garver et al (2016) and Marty et al (2014) were published, HSMI actually had been observed in BC, but this observation is not reported and none of you report HSMI in a PRV-free Atlantic salmon.

Going back to your paper in Nature, you are very specific that you are referring to the organ damage caused by HSMI: *"Consequently, if HSMI diagnosis is based solely on histopathological heart lesions which can occur in the absence of PRV, then PRV cannot be assumed to be the causative agent of the disease, but rather one of multiple stand-alone or synergistic putative factors."*

My confusion stems from papers published by the authors of your paper that state HSMI has not been found in BC and so I don't know where to find reporting that HSMI has been diagnosed without PRV.

It is confusing - HSMI lesions were recognized in BC farm salmon in 2008 by the Province of BC farm salmon audits, but in 2014 and 2016 it was reported that HSMI has not been observed in BC, and now it is stated that HSMI does occur in BC and sometimes without PRV, citing a paper that states that HSMI does not occur in BC.

I hope you can see my cause for confusion.

Another note of concern where you state that morbidity in farm salmon with heart lesions is "uncommon" you cite several unpublished works from 1990-1992, a time period when the industry was predominantly farming Pacific salmon, which are not known to develop HSMI. So looking for morbidity in Pacific salmon with heart lesions, does not appear to inform on morbidity in Atlantic salmon with HSMI. In my surveys, morbidity and PRV are common in BC farm salmon today (see attached). Can you supply the reports you cite in 23-26?

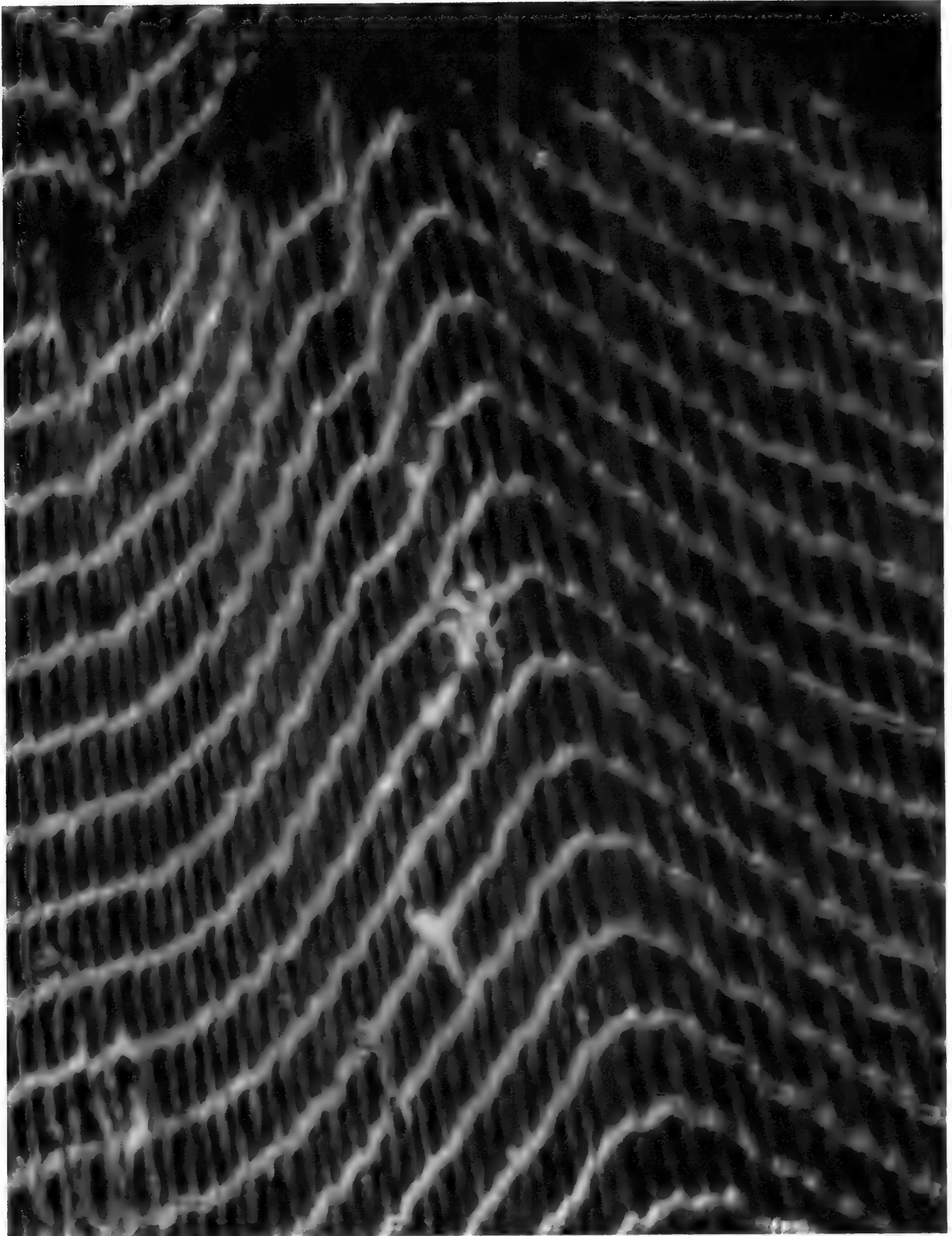
So two questions:

1. Which studies report HSMI in absence of PRV in Atlantic salmon?
2. Can you supply the unpublished reports cited as 23-26?

Thank you for your consideration of this important matter. I have copied others working on PRV in BC.



s.19(1)





Ministry of  
Agriculture

## Animal Health Centre

AAVLD - Accredited Laboratory

**Ministry of Agriculture**  
1767 Angus Campbell Road  
Abbotsford BC V3G 2M3  
Telephone : (604) 556-3003  
Facsimile: (604) 556-3010  
Toll-Free : 1-800-661-9903

### Interim Report AHC Case: 17-400

Last Updated: 01/26/17 1:38 PM

Pathologist: Gary D. Marty

Received Date: 01/24/17

Collected Date:

Client Ref No:

Veterinarian:

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner:

Phone:

Fax:

#### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

#### Case History

**Requesting description by Dr. Gary Marty of the difference between HSMI-like and HSMI lesions in BC farm salmon.**

**Addendum:** This case is the latest in a series of requests for information from G. Marty. An e-mail from Gary Marty (Monday, December 19, 2016 5:16PM) provides details on the questions to answer for this report:

"There is something I am not understanding here.

[You] excel in precise use of terms, but you say 'the BC disease does not behave like the Norwegian form of HSMI.'

That sounds like you are seeing a 'disease' not environmental damage.

When you report 'HSMHike' lesions are you saying:

- it looks like HSMI, but you are not sure? Have you consulted with experts on the disease?

- Or are you are saying there are small differences between what you are seeing and what the international community is calling HSMI and so again you are not sure.

So two questions:

1. What does 'HSMHike' mean?

2. And when you say 'the BC disease' what are you saying?"

s.19(1)

**\*All histories are copied verbatim from the submission form**

### Final Diagnosis

My answer to the specific questions about the relation of HSMI and disease is based, in part, on the dictionary definition

of disease:

"an impairment of the normal state of the living animal...or one of its parts that interrupts or modifies the performance of the vital functions, is typically manifested by distinguishing signs and symptoms, and is a response to environmental factors (as malnutrition, industrial hazards, or climate), to specific infective agents (as worms, bacteria, or viruses), to inherent defects of the organism (as genetic anomalies), or to combinations of these factors." Source: The Merriam-Webster website (<https://www.merriam-webster.com/dictionary/disease>, accessed 2017-01-26)

When the word "disease" is used by medical practitioners, it is commonly prefaced by the organ or potential cause of the disease. Infectious disease is only one category of disease; many other categories of disease are recognized, including: heart disease, genetic disease, autoimmune disease, and environmental disease. An Internet browser search for any of these terms will yield many subcategories for each term.

What does 'HSMHike' mean?

**GM response:** In this context, I use "HSMHike" to mean that the lesions in the heart and skeletal muscle of affected BC fish are similar to the lesions that have been described in the heart and skeletal muscle of Norwegian fish with HSMI. Stained tissue sections from fish that I have diagnosed as HSMHike have been sent to Norway for a second opinion. The feedback I received is that the Norwegian pathologists agree that the lesions in the BC fish are within the range of what they would diagnose as HSMI.

I have also received feedback from Norwegian salmon farm veterinarians. They commonly receive a diagnosis of HSMI from their pathologist, but in many cases the disease on their farm is like the BC form of the disease (i.e., a few fish die of the disease, but overall the population is eating well and growing well). Therefore, the diagnosis of HSMI is not particularly useful for Norwegian salmon farm veterinarians unless large numbers of fish on their farm are sick—then the diagnosis is useful to differentiate HSMI from other diseases.

And when you say 'the BC disease' what are you saying?"

**GM response:** The BC form of the disease affects a small proportion of the fish on the farm. In contrast, HSMI in Norway is described by Kongtorp et al. (2004) as, "Morbidity is high, as most fish in affected sea cages show histopathological lesions in heart and skeletal muscle. Mortality varies from almost insignificant up to 20%." Google defines morbidity as, "the condition of being diseased". Restated, Kongtorp et al. (2004) state that in farms with HSMI, most of the fish are sick, most of sick fish have abnormalities in their heart and skeletal muscle, and mortality varies from 0 – 20%.

The form of HSMI described by Kongtorp et al. (2004) has never been described in BC. I have never seen a case in which "most fish in affected sea cages show histopathological lesions in heart and skeletal muscle." As I wrote in response to the previous question, I am hearing that many Norwegian veterinarians are now seeing mild forms of HSMI on their farms that do not fit the original description of the disease as written by Kongtorp et al. (2004).

What is the difference between HSMHike and HSMI lesions in BC farm salmon?

**GM response:** There is no difference. Since 2008 I have diagnosed lesions in the heart (and more recently skeletal muscle) that are similar to lesions in fish from Norway with HSMI. [I diagnosed similar heart lesions before 2008, but I did not specifically note that those lesions were similar to HSMI until after I attended a continuing education seminar in early 2008.] The similarity of lesions in BC salmon to lesions in Norwegian salmon with HSMI was confirmed in 2016 by Dr. Kristi Miller's group and by Norwegian pathologists.

The difference is one of word usage. Different people use different words to summarize their findings. Terms that have been used for the disease in BC Atlantic salmon include: HSMI, HSMHike, idiopathic cardiomyopathy, and heart disease of unknown cause. Regardless of what we call the disease in BC, affected fish have the same lesions, morbidity (low), and mortality (low).

#### Literature Cited:

Kongtorp, R.T., A. Kjerstad, T. Taksdal, A. Guttvik, and K. Falk. 2004. Heart and skeletal muscle inflammation in Atlantic salmon, *Salmo salar* L.: a new infectious disease. J. Fish Dis. 27:351–358.

## Additional Tests

Results are Pending for Additional time to interpret/rspd to client, each 15mins

Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

These results relate only to the animals or items tested.

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unauthorized persons. If you have received the transmission in error, please contact the sender immediately by telephone.

**END OF REPORT**

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-----Original Message-----

From: DiCicco, Emiliano

Sent: Sat 5/21/2016 9:00 PM

To: Miller-Saunders, Kristi

Subject: I: HSMI diagnoses in BC

Hi... look at the following... [REDACTED]

[REDACTED]

I look forward to reading Hugh's reply, though...

Talk you soon,

Emiliano

\*\*\*\*\*

Emiliano Di Cicco DVM PhD

Fish Health Researcher

Molecular Genetics Lab - Pacific Biological Station Department of Fisheries and Oceans, Canada

3190 Hammond Bay Rd, Nanaimo, BC V9T 1K6 - Canada

Phone: office (250) 756 7045

Cell [REDACTED]

e-mail: Emiliano.DiCicco@dfo-mpo.gc.ca

s.19(1)

s.21(1)(a)

s.21(1)(b)

-----Messaggio originale-----

Da: Marty, Gary D AGRI:EX [mailto:Gary.Marty@gov.bc.ca]

Inviato: sab 21/05/2016 12.36

A: DiCicco, Emiliano; 'ferguson@fishpathology.com'

Oggetto: HSMI diagnoses in BC

Hi Hugh and Emiliano,

It was nice to see you at the meeting on Wednesday. I appreciate that conflict is a part of science. In this case, some additional information might help clarify some things.

In particular, I want to clarify how HSMI has been defined in BC since I began working in my current position in 2004. It was not long after I started that I began seeing occasional fish with epicarditis, endocarditis, and variable amounts of myocardial necrosis. When I first diagnosed those cases, I provided a general comment that these lesions were



consistent with systemic disease. In February 2008, [REDACTED] provided BC vets a continuing education session that summarized the pathology of emerging European diseases in farmed Atlantic salmon. When she showed images of HSMI, I immediately recognized the lesions as similar to what I had been seeing microscopically in some BC fish. However, the aquaculture veterinarians said that they were not seeing a clinical pattern that was consistent with Norwegian HSMI (all the Atlantic salmon companies have Norwegian connections, so I assume that they are well aware of the clinical signs of HSMI). Therefore, we decided that what I was seeing was probably not the same as Norwegian HSMI. We understood HSMI to be the name of a disease syndrome, and that characteristic clinical signs were needed for a diagnosis of HSMI (i.e., similar morphologic lesions without clinical signs did not warrant a diagnosis of HSMI). After that session, when I saw inflammatory heart lesions that were similar to HSMI, I started adding to my comments a note that the lesions were similar to lesions in Norwegian fish with HSMI, but that HSMI had never been seen in BC.

The expert report that I produced in an ongoing Canadian legal case provides a good example:

Public reporting: Affidavit of Dr. Gary D. Marty sworn October 30, 2013, in Morton v. Minister of Fisheries and Oceans et al, Federal Court No. T-789-13

21. Have you tested fish for PRV and/or HSMI with results that contradict the results of your testing for MHC?

I have not tested fish for PRV and/or HSMI with results that contradict the results of my testing for MHC, but I have tested fish in which the suite of lesions was different than the groups of fish I examined from MHC or DFO.

As described in my answer to question #4, among all the testing I have done for HSMI (e.g., the BC Fish Health Auditing and Surveillance Program), I occasionally diagnose "unexplained heart lesions" as the cause of death. However, the prevalence of PRV in tested cases (80%) is the same as the prevalence of PRV among (i) groups of fish that die of other causes and (ii) healthy fish that are sampled for pretransfer screening.

In two cases submitted directly by a BC fish farm company other than Marine Harvest (one case in 2011 and one case from a different farm in 2013), I diagnosed unexplained heart lesions as the cause of death in all of the fish in the sample group. These cases were not tested for PRV, but based on other data there is an 80% chance that they would be PRV positive. In this year's case, I requested a second submission that included skeletal muscle for histopathology (skeletal muscle is not included in routine submissions for diagnostic purposes). One of the 10 fish included in the second submission had severe heart lesions but no skeletal muscle inflammation; therefore, this fish did not have HSMI. Three other fish had moderate to severe heart lesions along with mild inflammation of skeletal muscle; therefore, these fish had inflammation of the heart and skeletal muscle, which are two features of HSMI. However, the farm's veterinarian told me that the fish did not have clinical signs consistent with the description of the European syndrome HSMI (see Dr. Nylund's expert report, answer to his question 24). Because these BC fish did not have all features of the European syndrome HSMI (i.e., clinical features are different), it is not appropriate to diagnose HSMI in these fish. Without consistent clinical signs, a diagnosis of HSMI in these fish is likely to result another example of the diagnostic "confusion" described by Dr. Nylund in his expert report (i.e., the response to his question 22). The submission form submitted with the second BC sample included a history that stated, "As environmental conditions improved, mortality

dropped significantly. Mortality is now low normal with no clinical signs of disease." The cause of the heart lesions in these fish remains unknown, but all the information I have better fits "transient adverse environmental conditions" (e.g., exposure to algal toxins) as the cause of disease rather than PRV. Also, if BC strains of PRV were causing HSMI, it is not plausible to have 80% of BC Atlantic salmon infected with PRV every year since 2006, but have only two cases of HSMI during that same period.

This expert report was entered into evidence and is available to the public. In the 2.5 years since I produced this document, I have not seen any information that compels me to change my response to this question (# 21). After our meeting on Wednesday, [REDACTED] informed me that the 2011 and 2013 cases in my expert report [REDACTED]

I think that the information above supports the conclusion that I diagnose inflammation in the heart and skeletal muscle when it occurs; however, I do not diagnose HSMI in these fish because the submitting veterinarians tell me that their fish do not have clinical signs consistent with HSMI. As a referral veterinarian, I would need some very strong justification to diagnose a syndrome contrary to the information provided by my referring veterinarians.

To summarize, I provided information in a public document 2.5 years ago that stated, "these fish had inflammation of the heart and skeletal muscle, which are two features of HSMI". My understanding from our meeting on Wednesday is that we do not disagree on these two features of HSMI. My understanding is that the fish I examined and the fish you examined were from the same farm and from the same outbreak. I reported "inflammation of the heart and skeletal muscle" publicly in 2013. Your findings of the same lesions from the same outbreak were reported yesterday (2.5 years later). [REDACTED] when I read things like the following in a CBC news report (emphasis mine):

"A feared viral disease proven deadly in Norwegian fish farms has been confirmed for the first time by federal scientists studying farmed salmon in B.C.

Heart and Skeletal Muscle Inflammation (HSMI) has been linked to the deaths of up to 20 per cent at some Norwegian farms.

'The concern is that it is a disease that hasn't previously been detected in B.C. and at the present time we really don't have sufficient evidence to know if it causes mortality or is a production issue here,' said Kristi Miller, part of a team of federal scientists studying farmed fish samples from sites along the B.C. coast."

<http://www.cbc.ca/news/canada/british-columbia/farmed-salmon-bc-disease-hsmi-aquaculture-1.3593958>

Best regards,

Gary

s.19(1)

s.20(1)(b)

P.S. In my experience, reporters will make changes to stories if errors are pointed out quickly. I recognize that the information I highlight from the CBC story was not included in the press release, and that it might have been influenced by discussions with other people (e.g., [REDACTED]).

-----  
Gary D. Marty, D.V.M., Ph.D., Diplomate, A.C.V.P.

Senior Fish Pathologist  
Animal Health Centre  
Ministry of Agriculture  
1767 Angus Campbell Rd.  
Abbotsford, BC, V3G 2M3  
604-556-3123

s.19(1)

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** March-15-19 10:32 AM  
**To:** [REDACTED]  
**Cc:** Polinski, Mark; [REDACTED] Miller-Saunders, Kristi; [REDACTED]  
DiCicco, Emiliano  
**Subject:** Re: Two Question on Paper on PRV in Nature

[REDACTED] Thank you for this. I couldn't help smiling. [REDACTED]  
[REDACTED]

On Thu, 14 Mar 2019 at 13:58, [REDACTED] wrote:  
Dear Dr. Mark Polinski:

Congratulations on your publication in the high impact journal Nature.

I am writing for clarification on your statement that HSMI has been diagnosed in BC salmon in absence of PRV.

In your section titled **Preface concerning the diagnosis of HSMI**; you rely on two papers in making the statement that HSMI has been reported in farm salmon in absence of PRV: "... *if HSMI diagnosis is based solely on histopathological heart lesions which can occur in the absence of PRV...*" The papers you appear to cite are Marty et al (2015) and Di Cicco et al (2018).

This is an important conclusion, an outlier to other literature on PRV in Atlantic salmon, and it is a "preface" to the work you report on in your paper.

However, Di Cicco et al (2018) say that they suspect that the one fish reported the farm audit data with heart lesions in absence of PRV "*was not an HSMI fish*".

Similarly, Marty et al (2015), state: "*None of the fish in our study had microscopic lesions diagnostic for HSMI.*" They noted heart damage, but suggest it was due to *Loma salmonae* infection, and did not view it as HSMI.

Neither of these papers support the conclusion that HSMI occurs in absence of PRV.

Similarly in 2016, yourself and your co-authors, reported that western North America is "*a region now considered endemic for PRV but without manifestation of HSMI*" (Garver et al 2016). This is a sweeping statement that includes all the farm salmon health audits, in all years and the private reports by Dr. Marty's lab to industry. This suggest that prior to 2016 HSMI was never seen in BC.

However, four months *after* Garver et al (2016) stated that HSMI does not manifest in BC, Dr Marty appears to reverse his position in an internal email that he did find HSMI-like lesions in BC farm salmon beginning in at least 2008 (attached).

When I asked Dr. Marty to describe the difference between HSMI and HSMI-like he said there was "no difference" (attached). Excuse me if I have this wrong, but this suggests that when Garver et al (2016) and

Marty et al (2014) were published, HSMI actually had been observed in BC, but this observation is not reported and none of you report HSMI in a PRV-free Atlantic salmon.

Going back to your paper in Nature, you are very specific that you are referring to the organ damage caused by HSMI: *"Consequently, if HSMI diagnosis is based solely on histopathological heart lesions which can occur in the absence of PRV, then PRV cannot be assumed to be the causative agent of the disease, but rather one of multiple stand-alone or synergistic putative factors."*

My confusion stems from papers published by the authors of your paper that state HSMI has not been found in BC and so I don't know where to find reporting that HSMI has been diagnosed without PRV.

It is confusing - HSMI lesions were recognized in BC farm salmon in 2008 by the Province of BC farm salmon audits, but in 2014 and 2016 it was reported that HSMI has not been observed in BC, and now it is stated that HSMI does occur in BC and sometimes without PRV, citing a paper that states that HSMI does not occur in BC.

I hope you can see my cause for confusion.

Another note of concern where you state that morbidity in farm salmon with heart lesions is "uncommon" you cite several unpublished works from 1990-1992, a time period when the industry was predominantly farming Pacific salmon, which are not known to develop HSMI. So looking for morbidity in Pacific salmon with heart lesions, does not appear to inform on morbidity in Atlantic salmon with HSMI. In my surveys, morbidity and PRV are common in BC farm salmon today (see attached). Can you supply the reports you cite in 23-26?

So two questions:

1. Which studies report HSMI in absence of PRV in Atlantic salmon?
2. Can you supply the unpublished reports cited as 23-26?

Thank you for your consideration of this important matter. I have copied others working on PRV in BC.



s.19(1)

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** March-16-19 3:17 PM  
**To:** Waddington, Zac; Niccolò Vendramin; Gagne, Nellie; Farrell, Anthony; Weber, Lily; Burgetz, Ingrid; Struthers, Alistair; 'Nathalie.N.Bruneau@inspection.gc.ca'; 'Myron.Roth@gov.bc.ca'; [REDACTED]; 'espen.rimstad@nmbu.no'; 'mark.powell@hi.no'; 'iagardner@upei.ca'; Garver, Kyle; Polinski, Mark; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED]; 'Gary.Marty@gov.bc.ca'; [REDACTED] Boily, France  
**Cc:** Olivier, Gilles; Craig Stephen; Parsons, Jay  
**Subject:** RE: Draft PRV SAR For Your Review  
**Attachments:** PRV CSAS SAR\_KM Comments.docx

My comments on the SAR are enclosed.

Kristi Miller

---

**From:** Waddington, Zac  
**Sent:** March 15, 2019 10:30 AM  
**To:** Niccolò Vendramin; Gagne, Nellie; Farrell, Anthony; Weber, Lily; Burgetz, Ingrid; Struthers, Alistair; 'Nathalie.N.Bruneau@inspection.gc.ca'; 'Myron.Roth@gov.bc.ca'; [REDACTED] Miller-Saunders, Kristi; [REDACTED] 'espen.rimstad@nmbu.no'; 'mark.powell@hi.no'; 'iagardner@upei.ca'; Garver, Kyle; Polinski, Mark; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED]; 'Gary.Marty@gov.bc.ca'; [REDACTED] Boily, France; Garver, Kyle  
**Cc:** Olivier, Gilles; Craig Stephen; Parsons, Jay  
**Subject:** RE: Draft PRV SAR For Your Review

Hello all,

My comments:

Line 31: "consequences to Fraser River Sockeye Salmon abundance and diversity..." At some point in the document it would be good to clarify that we were assessing all Fraser River sockeye stocks as a whole (i.e. not at the CU basis), and what the definition of "negligible" is as it relates to the degree of loss of abundance/diversity

Line 56: it might be worth making explicit that the detection of PRV-1 genetic material does not indicate viable, infective PRV-1 virus.

Line 117: Same comment as for line 56. Perhaps here would be a better place to make clear that the inability to culture the virus necessitates bioassays to determine the presence/absence of "live" infective PRV. Detection of genetic material is not synonymous with infectivity.

Line 243: "...beginning of June..."

Line 381: "...exposure time and dose required to result in a PRV-1 infection..."

Line 412: suggest clarifying that "salmon" refers to both farmed Pacific (Chinook) salmon and Atlantic salmon.

Line 467-469: I am not clear what is being said here, particularly "...including need for minimal standards of diagnostic tools." Suggest rewording to clarify.

Cheers,

s.19(1)

Zac

**From:** Niccolò Vendramin [mailto:niven@aqua.dtu.dk]

**Sent:** March-13-19 9:43 AM

**To:** Gagne, Nellie <Nellie.Gagne@dfo-mpo.gc.ca>; Farrell, Anthony <tony.farrell@ubc.ca>; Weber, Lily <Lily.Weber@dfo-mpo.gc.ca>; Burgetz, Ingrid <Ingrid.Burgetz@dfo-mpo.gc.ca>; Waddington, Zac <Zac.Waddington@dfo-mpo.gc.ca>; Struthers, Alistair <Alistair.Struthers@dfo-mpo.gc.ca>; 'Nathalie.N.Bruneau@inspection.gc.ca' <Nathalie.N.Bruneau@inspection.gc.ca>; 'Myron.Roth@gov.bc.ca' <Myron.Roth@gov.bc.ca>; [REDACTED] Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; [REDACTED] 'espen.rimstad@nmbu.no' <espen.rimstad@nmbu.no>; 'mark.powell@hi.no' <mark.powell@hi.no>; 'iagardner@upei.ca' <iagardner@upei.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Polinski, Mark <Mark.Polinski@dfo-mpo.gc.ca>; Mimeault, Caroline <Caroline.Mimeault@dfo-mpo.gc.ca>; Holt, Kendra <Kendra.Holt@dfo-mpo.gc.ca>; Johnson, Stewart <Stewart.Johnson@dfo-mpo.gc.ca>; Jones, Simon <Simon.Jones@dfo-mpo.gc.ca>; [REDACTED] 'Gary.Marty@gov.bc.ca' <Gary.Marty@gov.bc.ca>; [REDACTED] Boily, France <France.Boily@dfo-mpo.gc.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>

**Cc:** Olivier, Gilles <Gilles.Olivier@dfo-mpo.gc.ca>; Craig Stephen <cstephen@cwbc-rcsf.ca>; Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>

**Subject:** RE: Draft PRV SAR For Your Review

Dear all,

Thanks for the valuable effort in compiling all information from the meeting.

A couple of inputs from here.

1) Line 456 "Undertake an assessment of factors influencing risk of importing **exotic strains** of PRV into BC". Exotic strains for PRV is a definition that is too open/unprecise. Could it be amended as "avoid introduction of PRV-1 isolates with genetic markers increasing the risk of developing HSMI" and "avoid introduction of PRV genogroups other than PRV-1"

2) The following reference should be included in the reference list.

- Di Cicco E, Ferguson HW, Kaukinen KH, Schulze AD, Li S, Tabata A, Günther OP, Mordecai G, Suttle CA, and Miller KM. 2018. The same strain of Piscine orthoreovirus (PRV-1) is involved in the development of different, but related, diseases in Atlantic and Pacific Salmon in British Columbia. FACETS 3: 599–641. doi:10.1139/facets-2018-0008" this should be included.

3) if it can be of any help, we got recently published the paper of PRV-3 causing heart pathology in Rainbow trout.

- Vendramin N, Kannimuthu D, Olsen AB, Cuenca A, Teige LH, Wessel Ø, Iburg TM, Dahle MK, Rimstad E, Olesen NJ (2019) Piscine orthoreovirus subtype 3 (PRV-3) causes heart inflammation in rainbow trout (*Oncorhynchus mykiss*). Vet Res 50:14 . doi: 10.1186/s13567-019-0632-4

<https://veterinaryresearch.biomedcentral.com/articles/10.1186/s13567-019-0632-4>

Best regards

Niccolò and Espen

s.19(1)

**From:** Gagne, Nellie [mailto:Nellie.Gagne@dfo-mpo.gc.ca]

**Sent:** 12. marts 2019 20:48

**To:** Farrell, Anthony; Weber, Lily; Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair;

'Nathalie.N.Bruneau@inspection.gc.ca'; 'Myron.Roth@gov.bc.ca'; ' '; Miller-Saunders, Kristi; 'espen.rimstad@nmbu.no'; Niccolò Vendramin; 'mark.powell@hi.no'; 'iagardner@upei.ca'; Garver, Kyle; Polinski, Mark; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; 'Gary.Marty@gov.bc.ca'; ' '; Boily, France; Garver, Kyle  
**Cc:** Olivier, Gilles; Craig Stephen; Parsons, Jay  
**Subject:** RE: Draft PRV SAR For Your Review

A few comments/suggestion in addition:

Ln 48-50: the first sentence is about variation of virulence due to the virus itself, but the second could be about the genetic of the fish... is the first sentence correct? Or it should be ... variation in the virulence among strains of PRV-1 and/or among Atlantic Salmon of different origins.

Ln 108: due to PRV...

Ln 109: released ? (instead of transferred)

Ln 128 and 156: in conjunction

Ln 132: or any severe skeletal... (was there any skeletal inflammation, even mild?)

Ln 163: is it preliminary or the data is now analysed ?

Ln 172: cut the sentence (too long)

Ln 358 and 265 and 428: is minimal = throughout the paper I think the "negligible" label as in fig 3 is used, whereas here it says minimal

Ln 395: not consensus to change.. (grammar or rewording required)

All the best,  
Nellie

**From:** Farrell, Anthony <tony.farrell@ubc.ca>

**Sent:** March-08-19 2:59 PM

**To:** Weber, Lily <Lily.Weber@dfo-mpo.gc.ca>; Burgetz, Ingrid <Ingrid.Burgetz@dfo-mpo.gc.ca>; Waddington, Zac <Zac.Waddington@dfo-mpo.gc.ca>; Struthers, Alistair <Alistair.Struthers@dfo-mpo.gc.ca>; Gagne, Nellie <Nellie.Gagne@dfo-mpo.gc.ca>; 'Nathalie.N.Bruneau@inspection.gc.ca' <Nathalie.N.Bruneau@inspection.gc.ca>; 'Myron.Roth@gov.bc.ca' <Myron.Roth@gov.bc.ca>; ' '; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; 'espen.rimstad@nmbu.no' <espen.rimstad@nmbu.no>; 'niven@vet.dtu.dk' <niven@vet.dtu.dk>; 'mark.powell@hi.no' <mark.powell@hi.no>; 'iagardner@upei.ca' <iagardner@upei.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Polinski, Mark <Mark.Polinski@dfo-mpo.gc.ca>; Mimeault, Caroline <Caroline.Mimeault@dfo-mpo.gc.ca>; Holt, Kendra <Kendra.Holt@dfo-mpo.gc.ca>; Johnson, Stewart <Stewart.Johnson@dfo-mpo.gc.ca>; Jones, Simon <Simon.Jones@dfo-mpo.gc.ca>; 'tim.hewison@griegseafood.com'

; 'Gary.Marty@gov.bc.ca' <Gary.Marty@gov.bc.ca>; ' '; Boily, France <France.Boily@dfo-mpo.gc.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>

**Cc:** Olivier, Gilles <Gilles.Olivier@dfo-mpo.gc.ca>; Craig Stephen <cstephen@cwahc-rcsf.ca>; Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>

**Subject:** Re: Draft PRV SAR For Your Review

s.19(1)

All

Unless I comment otherwise below, I am accepting any suggested edits on the version sent to me.

The following specific minor comments are for clarity, conciseness and correctness.

All the best



Tony

L28: A 'single' uncertainty cannot have a range. Perhaps you mean the "contributing uncertainties"...

L33 was = were; "were discussed. Expert participants voiced different opinions on the ....

L36 This piece is awkwardly constructed and points 1 and 2 have an unbalance sentence structure. Try...

The main uncertainties were (1) the likelihood of infection of wild salmon PRV-1 from infected Atlantic salmon farms, and (2) the consequences to Sockeye Salmon. In the former case, uncertainty exists because of the lack of data to estimate the concentration of PRV-1 from infected Atlantic salmon farms, the exposure duration required for infection to occur and the minimum infectious dose for adult and juvenile Sockeye salmon. For the later, uncertainty exists in applicability laboratory studies to estimate consequences.

L47 the = the current

L53 replace with "... but with typically considerably lower..."

L59 replace with "... trails with juvenile...". See line 69

L60 "... of a disease ..."

L63 fresh water = freshwater

L70 reword to be more precise and concise

moderate lesions, without any fish mortality, clinical signs or anaemia.

L72 reword to be more precise and concise

In four independent laboratory challenge trials with juvenile Sockeye Salmon, high viral loads of PRV-1 were generated without any fish mortality, clinical signs or anaemia. The interpretation of the histopathology results (i.e., lesions) was inconclusive.

L78 can not = cannot

L121 "...more frequent detection in Coho ..."

L141 "... some farms in Norway..."

L267 "... Salmon and confirmed only transient..."

L468 "... including the need ..."

s.19(1)

**From:** "Weber, Lily" <[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca)>

**Date:** Thursday, March 7, 2019 at 5:43 AM

**To:** "Burgetz, Ingrid" <[Ingrid.Burgetz@dfo-mpo.gc.ca](mailto:Ingrid.Burgetz@dfo-mpo.gc.ca)>, "Waddington, Zac" <[Zac.Waddington@dfo-mpo.gc.ca](mailto:Zac.Waddington@dfo-mpo.gc.ca)>, "Struthers, Alistair" <[Alistair.Struthers@dfo-mpo.gc.ca](mailto:Alistair.Struthers@dfo-mpo.gc.ca)>, "Gagne, Nellie" <[Nellie.Gagne@dfo-mpo.gc.ca](mailto:Nellie.Gagne@dfo-mpo.gc.ca)>, "Nathalie.N.Bruneau@inspection.gc.ca" <[Nathalie.N.Bruneau@inspection.gc.ca](mailto:Nathalie.N.Bruneau@inspection.gc.ca)>, Myron Roth <[Myron.Roth@gov.bc.ca](mailto:Myron.Roth@gov.bc.ca)>, "Kristi Miller-Saunders" <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>, "espen.rimstad@nmbu.no" <[espen.rimstad@nmbu.no](mailto:espen.rimstad@nmbu.no)>, "niven@vet.dtu.dk"

<niven@vet.dtu.dk>, ""mark.powell@hi.no"" <mark.powell@hi.no>, ""iagardner@upei.ca""  
<iagardner@upei.ca>, Kyle Garver <Kyle.Garver@dfo-mpo.gc.ca>, "Polinski, Mark" <Mark.Polinski@dfo-mpo.gc.ca>, "Weber, Lily" <Lily.Weber@dfo-mpo.gc.ca>, "Mimeault, Caroline" <Caroline.Mimeault@dfo-mpo.gc.ca>, "Holt, Kendra" <Kendra.Holt@dfo-mpo.gc.ca>, "Johnson, Stewart" <Stewart.Johnson@dfo-mpo.gc.ca>, Simon Jones <Simon.Jones@dfo-mpo.gc.ca>, [REDACTED]  
[REDACTED] "Farrell, Anthony" <tony.farrell@ubc.ca>,  
[REDACTED] "Gary.Marty@gov.bc.ca"  
<Gary.Marty@gov.bc.ca>, [REDACTED]  
[REDACTED] "Boily, France" <France.Boily@dfo-mpo.gc.ca>, Kyle Garver <Kyle.Garver@dfo-mpo.gc.ca>  
Cc: "Olivier, Gilles" <Gilles.Olivier@dfo-mpo.gc.ca>, Craig Stephen <cstephen@cwahc-rsfc.ca>, "Parsons, Jay" <Jay.Parsons@dfo-mpo.gc.ca>  
**Subject:** Draft PRV SAR For Your Review

Dear Participants of the CSAS peer review on the risk to Fraser River Sockeye Salmon for PRV from Atlantic salmon farms in the Discover Islands area:

On behalf of the co-chairs (Gilles Olivier and Craig Stephen), attached please find the draft version of the Science Advisory Report (SAR) for the PRV risk assessment that we reviewed in January 2019.

We are seeking your comments and approval of this document. Recall that at the meeting we had agreed to the summary bullets, Recommendations and Other Considerations, so please focus your review on the other parts of the report including the Introduction, Analysis, Sources of Uncertainty, Conclusions, etc. to assess if there are any factual errors or omissions or other comments. However, you will note that there are a few changes to the summary bullets, recommendations and other considerations which were made to improve clarity and grammar. Please let us know if you agree with these changes.

We would appreciate receiving your comments by **Friday, March 22, 2019**. After which we will review all input, finalise the report, seek the chairs' and internal approvals, and format for posting on the DFO web site.

Thank you.

**Lily Weber**

Science Advisor, Aquaculture, Biotechnology and Aquatic Animal Health Science Branch  
Fisheries and Oceans Canada / Government of Canada

[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca) / Tel: [REDACTED]

Conseillère scientifique, Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques  
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Government  
of Canada

Gouvernement  
du Canada

Canada

s.16(2)(c)

s.19(1)

Line 26 The Likelihood Assessment concluded that at least one Fraser River Sockeye Salmon, at either the juvenile or adult stage, becoming infected with PRV-1 attributable to Atlantic salmon from in Discovery Islands area is very likely with high certainty.

Someone changed this to a range, but it was agreed upon at the meeting that there was high certainty that at least one sockeye salmon would become infected. There was no contention in the room about this likelihood, and the body of the document supports this.

Line 30 The Consequence Assessment concluded that the potential magnitude of consequences to Fraser River Sockeye Salmon abundance and diversity is negligible with reasonable certainty for juveniles and reasonable uncertainty for adults. The levels of the uncertainty of for this conclusion were discussed and expert participants came to different conclusions on the applicability, and abundance, and reliability of the data to support uncertainty estimates. A range of uncertainties between reasonable certainty to reasonable uncertainty were associated with this conclusion.

The uncertainty stemmed both from the abundance of data AND the applicability of challenge studies that are solely focused on the demonstration of mortality and clinical signs of diseases, something that has not been recapitulated in any PRV-challenge studies. I do not recall agreeing to "reasonable certainty" for juveniles. In the end we agreed on a range of uncertainties that did not divide juvenile and adult salmon into separate units.

Line 60 In laboratory challenge trials, in juvenile Atlantic or Sockeye Salmon, the high load of PRV-1 is not predicative of clinical signs of disease or mortality.

It is imperative that this statement be clarified as the pathological data was removed from this review

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Line 68 In the field, PRV-1 has been statistically associated with severe heart inflammation in farmed Atlantic Salmon and jaundice/anemia syndrome in farmed Chinook Salmon in British Columbia; in both cases, PRV was localized within the regions of tissue damage, but as these were field-based studies, causal relationship has not been established.

Line 71 In laboratory challenge trials with juvenile Atlantic Salmon, when high viral loads are generated, the BC variant of PRV-1 can cause minor to moderate lesions, but no fish mortalities nor clinical signs nor anaemia were observed. Clinical signs and mortality have also not been observed in PRV laboratory challenge studies in Norway, but a higher severity of lesions has been demonstrated.

As anaemia is not expected with HSMI, there is no reason to expect you would observe it in Atlantic salmon in a challenge study. It is imperative that one is honest that the lack of clinical signs and mortality in a challenge study is not unusual.

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Line 131 By contrast, in Pacific Canada, PRV-1 has failed to cause severe heart lesions or any severity of skeletal muscle inflammation following experimental challenge of Atlantic or Pacific salmon in Pacific Canada

Line 134 Additionally, the clinical signs of jaundice/anemia have not been successfully transmitted to naive Chinook, Coho or Sockeye salmon in laboratory challenge trials in Pacific Canada despite the successful passage and development of high-load PRV-1 blood infections

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Line 142 Pacific Canada, although PRV-1 is highly prevalent in farmed Atlantic Salmon and two subclinical-farm-level cases of HSMI-like disease have been suggested to date (Di Cicco et al., 2017; Polinski et al., 2019), clinical outbreaks of HSMI causing moderate levels mortality, as described in Norway, have never been reported.

The case of HSMI described in Di Cicco et al demonstrated both gross and pathological signs of the disease, and low level mortality in only a few net pens. It was not subclinical—as the vet on the farm sent samples for pathological investigation at the peak of the disease due to elevated mortalities and behavioural changes in the fish—it did not go unnoticed, as would be expected if it were truly subclinical.

Line 159 Sockeye Salmon injected with PRV developed considerable blood and kidney PRV loads but no weight loss or, morbidity or pathology could be attributed to the virus, and pathology was inconclusive (Polinski et al., 2016). Despite high prevalence and persistence of PRV in blood and kidney of Sockeye Salmon cohabitated with PRV positive Atlantic Salmon, no microscopic lesions, disease or mortality could be attributed to the virus, but again, pathology was inconclusive (Garver et al., 2016a). Preliminary data indicate that PRV infections in the absence of disease are inconsequential to Sockeye Salmon respiratory function (see Polinski and Garver, in review). To date, there is no evidence that PRV causes disease in Sockeye Salmon despite successful infection with the virus under experimental conditions (Garver et al., 2016a; Garver et al., 2016b; Polinski et al., 2016).

As pointed out in the meeting, not a single one of these studies on sockeye salmon took sufficient tissues for histopathological evaluation to statistically determine if there were lesions of significance. It is entirely untrue to say that there were “No microscopic lesions”, and there was no way to attribute what was observed to the virus due to lack of controls. Throughout this review, conclusions based on pathology should be clarified as being inconclusive. Even Gary Marty agreed to this. Moreover, in one of their studies, there were actually mortalities with fish dying of lesions that were not inconsistent with the anticipated disease (as mentioned by Dr. Marty in the meeting), but these were ignored in the paper, again due to lack of controls.

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Line 195 positive by approximately three to four months (Di Cicco et al., 2017), or 100 to 200 days, independent of location or time of stocking (Laurin et al. 2019; Polinski and Garver, unpublished data).

Line 259 PRV-1 infections on Atlantic Salmon farms in BC take several months between initial infection and 100% infection prevalence (Di Cicco et al., 2017; Laurin et al. 2019; Polinski and Garver unpublished data).

Line 266 Further, a summary of recent telemetry studies (Rechisky et al., 2018) reported observations with tagged Sockeye Salmon suggesting transient interactions with farm infrastructure when farms are fallowed.

Line 310 Despite successful infection with the virus, no weight loss, no change in the ratio of volume of red blood cells to blood, no anemia, no consequences to respiratory functioning, no consistent tissue lesions, and no mortality could be attributed to the

There was no formal assessment of consistency in tissue lesions possible given the fact that adequate controls were not taken, different tissues were examined in different studies, and the timing of tissue sampling varied. The lack of rigor and consistency associated with the pathological evaluations renders them inconclusive.

Line 316 Assuming that results from laboratory studies on the impact of PRV-1 infection in juvenile Sockeye Salmon are indicative of what occurs in the marine environment, it was concluded with [REDACTED] that the potential magnitude of consequences to Fraser River Sockeye Salmon abundance and diversity would be negligible.

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I do not recall agreeing that this was of reasonable certainty. I believe that the conclusion for juvenile and adult salmon ranged from reasonable uncertainty to reasonable certainty.

Line 321 To date, the only study reporting on the impacts of PRV-1 infection in adult Sockeye Salmon indicated that while infection with PRV-1 in the marine environment was associated with migratory losses as fish entered the river for one stock, the odds ratio of mortality to spawning grounds between infected and uninfected individuals was not significant ~~did not significantly affect the odds of dying before reaching spawning grounds in two Fraser River Sockeye Salmon stocks~~ (Miller et al., 2014).

No information has been removed or severed from this page

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** March-16-19 3:52 PM  
**To:** Weber, Lily; Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair; Gagne, Nellie; 'Nathalie.N.Bruneau@inspection.gc.ca'; 'Myron.Roth@gov.bc.ca'; [REDACTED]; Miller-Saunders, Kristi; [REDACTED]; 'espen.rimstad@nmbu.no'; 'niven@vet.dtu.dk'; 'mark.powell@hi.no'; 'iagardner@upei.ca'; Garver, Kyle; Polinski, Mark; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED]; 'tony.farrell@ubc.ca'; [REDACTED]; 'Gary.Marty@gov.bc.ca'; [REDACTED]; Boily, France; Garver, Kyle  
**Cc:** Olivier, Gilles; Craig Stephen; Parsons, Jay  
**Subject:** Re: Draft PRV SAR For Your Review  
**Attachments:** Comments on PRV risk assessment SAR.docx

All:

I was in the process of reviewing this document and I have a concern.

I know that we agreed to the summary bullet points and at this time they seem to be inviolate. However, some new information has come to light that suggests we might want to review the bullets and perhaps the findings themselves.

I do note that at line 42-43 SAR it is stated that this risk assessment was informed by a summary of the current state of knowledge related to PRV. But since this is a draft and has not yet been finalized there should be room for change should new information come to light.

I recall that during our meeting we discussed at some length whether PRV-1 caused any lesions on lab-tested fish and the ultimate answer was PRV-1 can cause minor to moderate lesions (in challenged Atlantic salmon) but no clinical signs of disease were observed (see lines 69-70 SAR)

However, I note that in a recently published paper:

### **Zhang et. al. 2019: High-Load Reovirus Infections Do Not Imply Physiological Impairment in Salmon**

The authors state that in lab tests with Atlantic salmon mild heart and/or skeletal muscle inflammation (that would be HSMI) **was diagnosed** in PRV-infected fish. And while it is true that some blood control fish exhibited mild heart inflammation it is incorrect to conclude that such lesions did not exist.

That said, here and attached are my comments (taking this into account):

s.19(1)

Note the words that are bolded below are the suggested changes. Words or phrases that are both bolded and underlined are emphasized for comment and consideration.

Line 20 – should read “ ...continuous shedding of **the virus** from farms, ..”[ed note: “... continuous shedding from farms, ..” really means little]

Lines 31 – leave as is using “would be” ...“is” is too certain a term to be applied given the uncertainties expressed in this assessment.

Line 40 - should read “...“consequences to **adult** sockeye salmon”. There were no laboratory data or proxies used to estimate or assess consequences to adult salmon.

Line 57 – “..... High loads of PRV-1 have been reported in **juvenile** Atlantic and Sockeye salmon **that were infected following intraperitoneal injection with PRV contaminated blood.**”

- There was no evidence presented to show that Atlantic salmon on farms or wild sockeye salmon exhibited “high loads” of the virus. We were only presented with evidence that showed prevalence of viral detections in farmed fish, hatcheries and wild fish.

Line 59 – “In laboratory challenge trials **using juvenile Atlantic salmon or juvenile Sockeye Salmon**, the high load of PRV-1 **was** not predicative of disease state.”

Line 62 – “... PRV-1 in seawater, although **the virus has been detected** in juvenile **sockeye** salmon in fresh water [Ed note: See Tables 3 and 4, working paper #1. These data are *only for sockeye salmon* at all life stages. There are no data shown for other species.

Table 1 does speak to other species but does not distinguish between ocean or fresh water stages. Also, these data only show detections of the virus (presence and or prevalence) ... there is nothing to suggest the fish are “infected” with the virus]

Line 64 - **Juvenile** Sockeye Salmon appear less susceptible to infection relative to **juvenile** Atlantic Salmon in British

Line 70 – 71 – “generated, it **would appear** that the BC variant of PRV-1 **may** cause minor to moderate **heart and/or skeletal muscle inflammation**, but no fish mortalities nor anaemia were observed.”

[Ed note: This language is more accurate following the publication of Zhang e. al. 2019. The authors report that PRV infected fish were diagnosed with heart and/or skeletal muscle inflammation. This is new information and since this (the SAR) is a draft report, it should more accurately reflect the current state of the science]

Line 90 – “This advisory report summarizes the advice developed during the January 28-30,”

[Ed note: delete the word “**consensus**” as there was no true consensus]

Lines 120 – 125 and throughout the report – I think we should be very careful in distinguishing between presence and prevalence of the virus and the term “infected”. Upon reflection there are no data or reports showing that wild salmon are “infected” with the virus, just that the virus was detected in wild fish populations. Detection does not equate to “infection”. Infection is the invasion of an organism's body tissues by disease-causing agents, their multiplication, and the reaction of host tissues to the infectious agents and the toxins they produce.

([https://journals.lww.com/pccmjournal/Citation/2015/09000/Detection\\_Versus\\_Infection\\_What\\_Is\\_the.15.aspx](https://journals.lww.com/pccmjournal/Citation/2015/09000/Detection_Versus_Infection_What_Is_the.15.aspx))



Detection Versus Infection; What Is the Difference?\* : Pediatric Critical Care Medicine

[journals.lww.com](https://journals.lww.com)

An abstract is unavailable.

Lines 123 – 125 – Again this summary point does not distinguish between juvenile and adult salmon (either Atlantic or sockeye). The studies referred to only looked at PRV intensity and infection in **juvenile** salmon (conformed during the peer review meeting by the authors) and it should be made clear that is the case.



Lines 131 – 133 – 131 – “By contrast, in Pacific Canada, PRV-1 has failed to cause severe heart lesions or **any severity of skeletal muscle inflammation** following experimental challenge of Atlantic or Pacific..”

[Ed Note: This is inaccurate. Zhang e.al. 2019 report that indeed PRV infected fish exhibited **both mild and moderate** heart and skeletal muscle lesions (HSMI). Also, in the draft working papers we reviewed we were advised that these papers which were referenced in the Peer review and which are now being cited as “published”, were “in press”. There is a distinct difference in those connotations. Given that these papers are now published the findings therein should be reflected in this document]

Lines 321 – 324 - As I read Miller et.al. 2014 it says: “While our case studies also take an association-based approach, the study merging acoustic tracking with microparasite monitoring was able to directly associate specific microbes with migration success, resolving two infectious agents, a microsporidian parasite (L. salmonae) and a virus (PRV), that were **correlated with premature migration mortality ...**” AND “Survivorship analysis was additionally performed based on L. salmonae and PRV positives and negatives, **with both microparasites significantly associated with migration losses ..**” so I fail to see how one can say that PRV does not significantly affect the odds of dying before reaching the spawning grounds.

Lines 447 – 448 - 447 example, determining shedding rates from PRV-1-infected salmon, and assessing prevalence of PRV-1 in Fraser River Sockeye Salmon through **active** surveillance, **especially in returning adult salmon**. [ED Note several of the scientists that supported this recommendation strongly hinted that examining prevalence of PRV in returning salmon was highly important]

Final comment: On page 11 there are footnoted statements that relate to the footnotes at the bottom of the page which read: “See dissenting opinion section below”

There is no Dissenting Opinion Section included on this draft. Will we see one before this document is completed?

EOF

**To:** Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair; Gagne, Nellie; 'Nathalie.N.Bruneau@inspection.gc.ca'; 'Myron.Roth@gov.bc.ca'; [REDACTED] Miller-Saunders, Kristi; [REDACTED] 'espen.rimstad@nmbu.no'; 'niven@vet.dtu.dk'; 'mark.powell@hi.no'; 'iagardner@upei.ca'; Garver, Kyle; Polinski, Mark; Weber, Lily; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED] 'tony.farrell@ubc.ca'; [REDACTED] 'Gary.Marty@gov.bc.ca'; [REDACTED] Boily, France; Garver, Kyle  
**Cc:** Olivier, Gilles; Craig Stephen; Parsons, Jay  
**Subject:** Draft PRV SAR For Your Review

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We are seeking your comments and approval of this document. Recall that at the meeting we had agreed to the summary bullets, Recommendations and Other Considerations, so please focus your review on the other parts of the report including the Introduction, Analysis, Sources of Uncertainty, Conclusions, etc. to assess if there are any factual errors or omissions or other comments. However, you will note that there are a few changes to the summary bullets, recommendations and other considerations which were made to improve clarity and grammar. Please let us know if you agree with these changes.

We would appreciate receiving your comments by **Friday, March 22, 2019**. After which we will review all input, finalise the report, seek the chairs' and internal approvals, and format for posting on the DFO web site.

Thank you.

**Lily Weber**

Science Advisor, Aquaculture, Biotechnology and Aquatic Animal Health Science Branch  
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Canada

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## Comments of PRV risk assessment SAR

Comments submitted by [REDACTED] David Suzuki Foundation

Note the words that are bolded below are the suggested changes. Words or phrases that are both bolded and underlined are emphasized for comment and consideration.

Line 20 – should read “ ....continuous shedding of **the virus** from farms, ..”[ed note: “... continuous shedding from farms, ..” really means little]

Lines 31 – leave as is with “would be” ...“is” is too certain a term to be applied given the uncertainties

Line 40 - should read ....“consequences to **adult** sockeye salmon”. There were no laboratory data or proxies used to estimate or assess consequences to adult salmon.

Line 57 – “..... High loads of PRV-1 have been reported in **juvenile** Atlantic and Sockeye salmon that were infected following intraperitoneal injection with PRV contaminated blood.” - There was no evidence presented to show that Atlantic salmon on farms or wild sockeye salmon exhibited “high loads” of the virus. We were only presented with evidence that showed prevalence of viral detections in farmed fish, hatcheries and wild fish.

Line 59 – “In laboratory challenge trials **using juvenile Atlantic salmon or juvenile Sockeye Salmon**, the high load of PRV-1 **was** not predicative of disease state.”

Line 62 – “... PRV-1 in seawater, although **the virus has been detected** in juvenile **sockeye** salmon in fresh water [Ed note: See Tables 3 and 4, working paper #1. These data are *only for sockeye salmon* at all life stages. There are no data shown for other species.

Table 1 does speak to other species but does not distinguish between ocean or fresh water stages. Also, these data only show detections of the virus (presence and or prevalence) ... there is nothing to suggest the fish are “infected” with the virus]

Line 64 - **Juvenile** Sockeye Salmon appear less susceptible to infection relative to **juvenile** Atlantic Salmon in British

Line 70 – 71 – “generated, **it would appear that** the BC variant of PRV-1 **may** cause minor to moderate **heart and/or skeletal muscle inflammation**, but no fish mortalities nor anaemia were observed.”

[Ed note: This language is more accurate following the publication of Zhang e. al. 2019. The authors report that PRV infected fish were diagnosed with heart and/or skeletal muscles inflammation. This is new information and since this is a draft report, it should more accurately reflect the current state of the science]

Line 90 – “This advisory report summarizes the advice developed during the January 28-30,”

[Ed note: delete the word “consensus” as there was no true consensus]

Lines 120 – 125 and throughout the report – I think we should be very careful in distinguishing between presence and prevalence of the virus and the term “infected”. Upon reflection there are no data or reports showing that wild salmon are “infected” with the virus, just that the virus was detected in wild fish populations. Detection does not equate to “infection”. Infection is the invasion of an organism's body tissues by disease-causing agents, their multiplication, and the reaction of host tissues to the infectious agents and the toxins they produce.

([https://journals.lww.com/pccmjournal/Citation/2015/09000/Detection\\_Versus\\_Infection\\_What\\_Is\\_the.15.aspx](https://journals.lww.com/pccmjournal/Citation/2015/09000/Detection_Versus_Infection_What_Is_the.15.aspx))

Lines 123 – 125 – Again this summary point does not distinguish between juvenile and adult salmon (either Atlantic or sockeye). The studies referred to only looked at PRV intensity and infection in **juvenile** salmon (conformed during the peer review meeting by the authors) and it should be made clear that is the case.

Lines 131 – 133 – “By contrast, in Pacific Canada, PRV-1 has failed to cause severe heart lesions or **any severity of skeletal muscle inflammation** following experimental challenge of Atlantic or Pacific..”

This is inaccurate. Zhang e.al. 2019 report that indeed PRV infected fish exhibited **both mild and moderate** heart and skeletal muscles lesions. Also, in the draft working papers we reviewed we were advised that these papers, which are now being cited as “published”, were “in press”. There is a distinct difference in those connotations. Given that these papers are now published the findings therein should be reflected in this document

Lines 321 – 324 - As I read Miller et.al. 2014 it says “While our case studies also take an association-based approach, the study merging acoustic tracking with microparasite monitoring was able to directly associate specific microbes with migration success, resolving two infectious agents, a microsporidian parasite (*L. salmonae*) and a virus (PRV), that were **correlated with premature migration mortality...**” AND “Survivorship analysis was additionally performed based on *L. salmonae* and PRV positives and negatives, **with both microparasites significantly associated with migration losses ..**” so, I fail to see how one can say that PRV does not significantly affect the odds of dying before reaching the spawning grounds. This should be either corrected or deleted.

Lines 447 – 448 - 447 example, determining shedding rates from PRV-1-infected salmon, and assessing prevalence of PRV-1 in Fraser River Sockeye Salmon through **active** surveillance, **especially in returning**

**adult salmon.** [ED Note several of the scientists that supported this recommendation strongly hinted that examining prevalence of PRV in returning salmon was highly important]

Final comment: On page 11 there are footnoted statements that related to the footnotes at the bottom of the page which read: "See dissenting opinion section below"

There is no Dissenting Opinion Section included on this draft. Will we see ne before this draft is finalized?

EOF

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## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** March-18-19 10:06 AM  
**To:** Withler, Ruth  
**Subject:** RE: PRV / pathogen framework workshops - wednesday and Friday

I will be there for wed. This I believe is really about at what level of stock aggregate these risk assessments should be geared to. I think one thing they have not previously considered is the at risk status of stocks that could be exposed.

---

**From:** Withler, Ruth  
**Sent:** March 18, 2019 9:58 AM  
**To:** Miller-Saunders, Kristi  
**Subject:** FW: PRV / pathogen framework workshops - wednesday and Friday

If you have any 'pre-prepared' material that you would like supported, you can send it to us ahead of time so we can 'pre-prepared' as well. I am assuming that you will be able to be on a call for the meetings?

R

**From:** MacDougall, Lesley  
**Sent:** March-18-19 9:56 AM  
**To:** Lowe, Geoff; Garver, Kyle; Jones, Simon; Miller-Saunders, Kristi; Beacham, Terry; Withler, Ruth  
**Subject:** PRV / pathogen framework workshops - wednesday and Friday

Hello all;

As you may have noted there are 2 workshops scheduled for this week – these are intended to advance our response to the recent 'Namgis / Morton court decision.

As you may know the court ruling was deferred to June to allow DFO to review the decision and adjust its current practices as required based on the decision and analysis.

In particular, two key questions for science have been articulated

- 1) how do we determine an appropriate aggregate? What should be the level of resolution, and what criteria need to be used, acknowledging that the size of aggregate will likely need to change based on amount and quality of data that exists for the species / population in question
- 2) what advice does science have to add to the definition of 'acceptable harm'?

on Wednesday, we will be looking to clarify what data, information, and tools do we have immediately available to assist in answering the questions posed, and if things go really well, look to apply available data to start scoping out appropriate criteria for defining aggregates, and looking at what some of the consequences might be.

On Friday we hope to extend the work from Wednesday to further application of data and criteria to flesh out a draft decision tree or framework that articulates the criteria and decision making, along with agreed-on method to define aggregates.

Your attendance is requested both on Wednesday and on Friday. I expect that the main focus will be on PRV at first, but the intent is to develop criteria and definitions, and a decision framework, that could be applied to other pathogens of interest as well. It's likely that the completed risk assessments will help to inform the discussion but you'll also be asked to update on new work and help guide development of decision framework based on what info we currently have even if it's not published yet.

Lesley MacDougall

A/Division Manager, Aquatic Diagnostics, Genomics & Technology / Division des diagnostics, la génomique, de la technologie aquatique Fisheries and Oceans Canada / Pêches et Océans Canada Pacific Biological Station / Station Biologique du Pacifique Nanaimo, B.C. V9T 6N7

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Lesley.MacDougall@dfo-mpo.gc.ca

No information has been removed or severed from this page

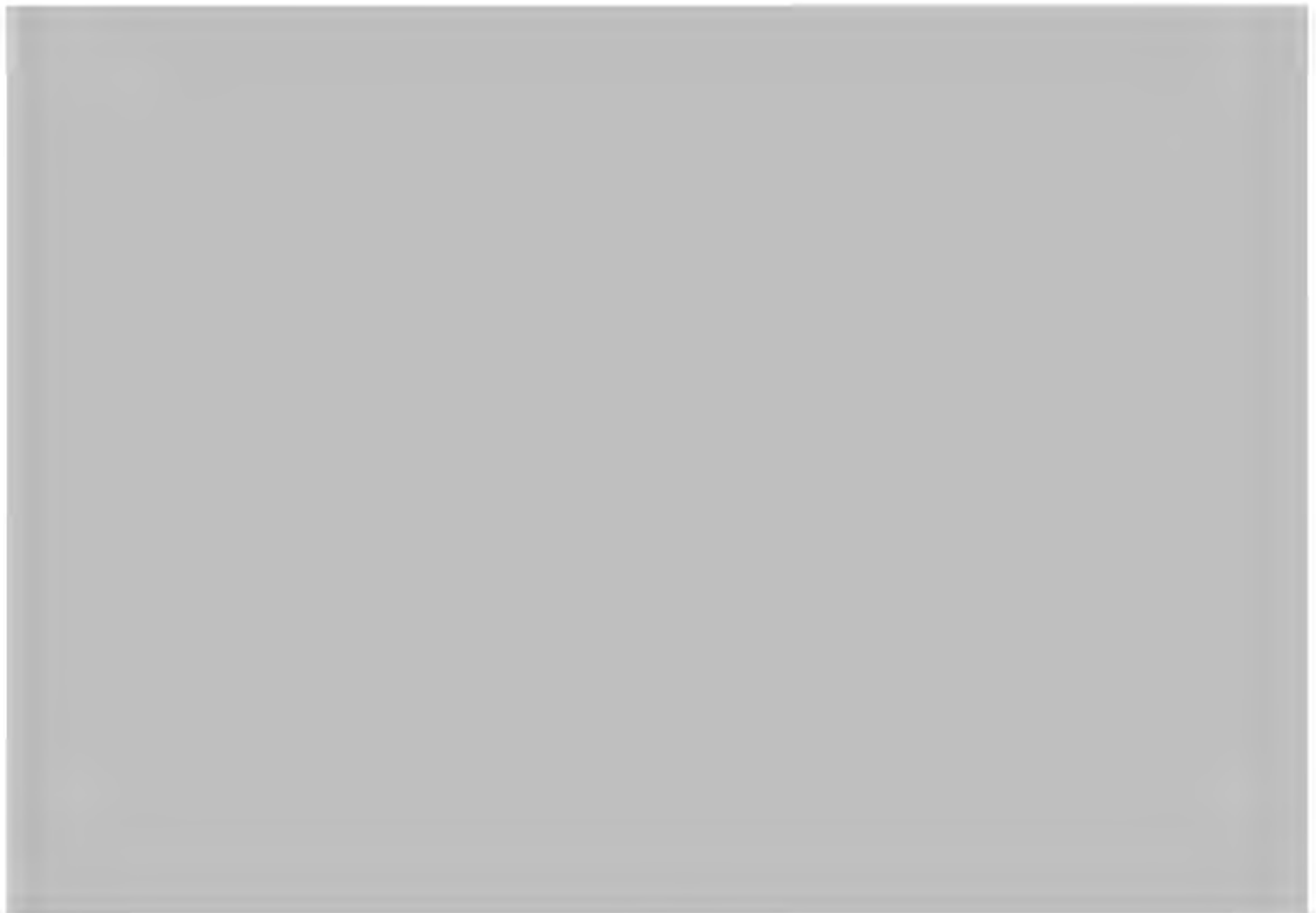
## Miller-Saunders, Kristi

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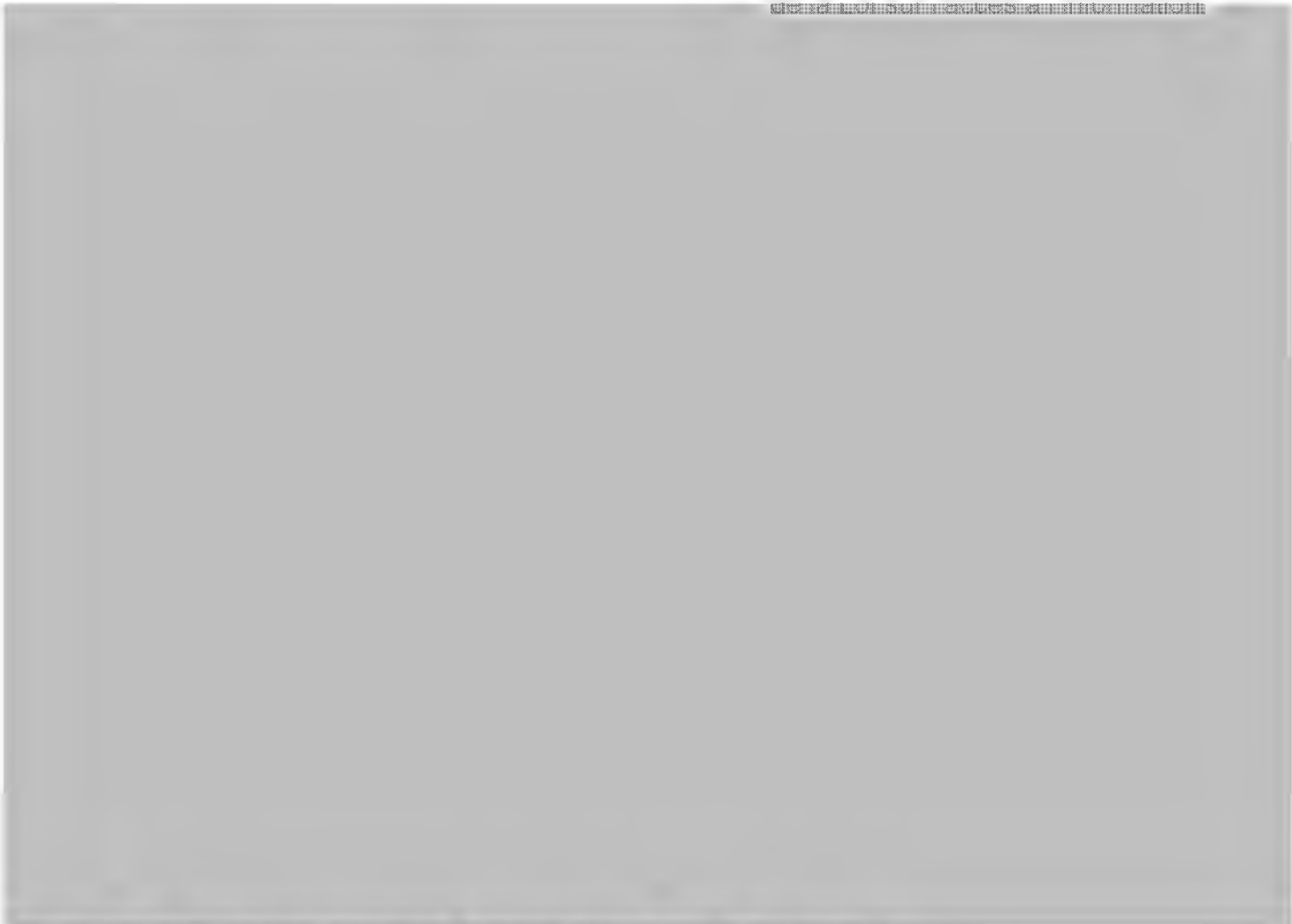
**From:** [REDACTED]  
**Sent:** March-27-19 2:55 PM  
**To:** Farrell, Anthony  
**Cc:** [REDACTED]; Weber, Lily; Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair; Gagne, Nellie; Nathalie.N.Bruneau@inspection.gc.ca; Myron.Roth@gov.bc.ca; [REDACTED]; Miller-Saunders, Kristi; [REDACTED]; espen.rimstad@nmbu.no; niven@vet.dtu.dk; mark.powell@hi.no; jagardner@upei.ca; Garver, Kyle; Polinski, Mark; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED]; [REDACTED]; Gary.Marty@gov.bc.ca; [REDACTED]; Boily, France; Olivier, Gilles; Craig Stephen; Parsons, Jay; Yangfan Zhang  
**Subject:** Re: [REDACTED] Zhang et al. 2019 in Frontiers in Physiology

OK ... [REDACTED]

Thanks Tony.









On Mar 27, 2019, at 10:50 AM, Farrell, Anthony <[tony.farrell@ubc.ca](mailto:tony.farrell@ubc.ca)> wrote:

All

In response to the email that I sent to all of you concerning Zhang et al. (2019),



Ultimately, the whole point of the scientific method is that research can be replicated and checked. If our m/s generated questions, then we as the authors must deal with the questions. This is very normal in my scientific life, especially at conferences and workshops where full dialogue is possible. Similarly, it is normal for me to powerfully defend what I believe in.

Whenever I have questions or concerns, I typically pick up a phone to have a real conversation, especially when students are having troubles. Conversation is critical to properly communicate.

The good thing, which is the big picture here I guess, is that the CSAS process for the PRV review had a full 2-day conversation to air matters fully. For that reason, I am happy not to meddle with the fundamentals of our CSAS conclusions, other than minor editorial changes.

All the best

Tony

PS After just over a week, Zhang et al. had been 'read' >1000 times and downloaded >100 times. Perhaps this speaks to the power of open access more than the content, but who knows?

s.19(1)

## Polinski, Mark

**From:** Miller-Saunders, Kristi  
**Sent:** Thursday, March 28, 2019 1:15 PM  
**To:** Weber, Lily; [REDACTED]  
**Cc:** Olivier, Gilles; Craig Stephen; Parsons, Jay; Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair; Gagne, Nellie; 'Nathalie.N.Bruneau@inspection.gc.ca'; 'Myron.Roth@gov.bc.ca'; [REDACTED] 'espen.rimstad@nmbu.no'; 'niven@vet.dtu.dk'; 'mark.powell@hi.no'; 'iagardner@upe.ca'; Garver, Kyle; Polinski, Mark; Mimeault, Caroline; Johnson, Stewart; Jones, Simon; [REDACTED] 'tony.farrell@ubc.ca'; [REDACTED] 'Gary.Marty@gov.bc.ca'; [REDACTED] Holt, Kendra; [REDACTED]; Boily, France; Garver, Kyle  
**Subject:** RE: Draft PRV SAR For Your Review

The problem I see is that in its communication on the results of this CSAS process, DFO has not always adequately reflected the uncertainties in the declaration of minimal risk, and the threshold that minimal risk was measured against. It is equally important that all communications make it clear the scope of this review—sockeye salmon ONLY and their risks pertaining to viral transmission ONLY in Discovery Islands.

Kristi

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
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s.19(1)

**From:** Weber, Lily  
**Sent:** March-28-19 10:44 AM  
**To:** [REDACTED]  
**Cc:** Olivier, Gilles <Gilles.Olivier@dfo-mpo.gc.ca>; Craig Stephen <cstephen@cwahc-rcsf.ca>; Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>; Burgetz, Ingrid <Ingrid.Burgetz@dfo-mpo.gc.ca>; Waddington, Zac <Zac.Waddington@dfo-mpo.gc.ca>; Struthers, Alistair <Alistair.Struthers@dfo-mpo.gc.ca>; Gagne, Nellie <Nellie.Gagne@dfo-mpo.gc.ca>; 'Nathalie.N.Bruneau@inspection.gc.ca' <Nathalie.N.Bruneau@inspection.gc.ca>; 'Myron.Roth@gov.bc.ca' <Myron.Roth@gov.bc.ca>; [REDACTED] Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; [REDACTED] 'espen.rimstad@nmbu.no' <espen.rimstad@nmbu.no>; 'niven@vet.dtu.dk' <niven@vet.dtu.dk>; 'mark.powell@hi.no' <mark.powell@hi.no>; 'iagardner@upe.ca' <iagardner@upe.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Polinski, Mark <Mark.Polinski@dfo-mpo.gc.ca>; Mimeault, Caroline <Caroline.Mimeault@dfo-mpo.gc.ca>; Johnson, Stewart <Stewart.Johnson@dfo-mpo.gc.ca>; Jones, Simon <Simon.Jones@dfo-mpo.gc.ca>; [REDACTED] [REDACTED] 'tony.farrell@ubc.ca' <tony.farrell@ubc.ca>; [REDACTED] 'Gary.Marty@gov.bc.ca' <Gary.Marty@gov.bc.ca>; [REDACTED] Holt, Kendra <Kendra.Holt@dfo-mpo.gc.ca>; [REDACTED]

[REDACTED]; Boily, France <France.Boily@dfo-mpo.gc.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>

**Subject:** RE: Draft PRV SAR For Your Review

[REDACTED]

On behalf Gilles Oliver, co-chair:

I appreciate your thorough review, and your comments and suggestions are being taken into consideration and we are working towards finalizing the SAR now that we have received comments on the summary advice document from all of the participants.

To specifically address a couple of the comments you raised, our apologies for omitting to disseminate that the CSAS office advised us that the Dissenting Opinion section was discontinued a few years ago and therefore this is no longer how CSAS Science Advisory Reports capture differences of opinion or advice. The CSAS office has confirmed that the current standard for capturing this important aspect of the meeting conclusions is within the "Uncertainties" section (which is where we have captured the differences in the uncertainty categorization that were discussed during the meeting). Additionally, the discussions underpinning these differences will be highlighted in the proceedings.

The recently published Zhang et al., 2019 paper was discussed during the meeting and is referenced within the PRV characterization paper and risk assessment and was available to the participants as an *in press* paper for their input and review.

The Science Advisory Report (SAR) is a summary of the consensus that was reached during the peer-review meeting. We will address your comment about consensus in the SAR. We will make reference to the CSAS definition of consensus (CSAS Policy on the Principle of Consensus (<http://www.dfo-mpo.gc.ca/csas-sccs/process-processus/consensus-eng.html>): "consensus means an absence of opposition to the meeting conclusions and advice that are based on scientific data and information and not on external considerations such as the potential impacts of future decisions....In many cases, some of the participants believe that additional data or more thorough analyses could support another conclusion or refine the conclusions and advice; however, they do not oppose the proposed conclusions, as these are supported by the current data and scientific analyses being considered". And we will reference the nature of the consensus and the area of disagreement.

The SAR includes the summary bullets that had been agreed to and includes the literature and data that were used to come to the conclusions for which the participants arrived at the meeting. The SAR is based on the discussions during the peer-review meeting and the two working papers that were thoroughly reviewed during the process. During the peer-review meeting there were opposing or differing opinions expressed only in regards to the uncertainty levels, which was captured in the collectively agreed on text found in the summary and reflected in the uncertainties section. While we appreciate you coming forward with your concerns following further reflection, the summary bullets were all agreed to as part of the peer-review meeting and as agreed, will not be changed other than for copy editing/grammatical purposes. The analysis and conclusions within the SAR need to be consistent with what was discussed and peer-reviewed during the meeting, otherwise they will do not reflect the peer-review that we collectively completed.

Regarding your comment on one particular assumption used during this process, again please be reminded that all assumptions were thoroughly discussed during the meeting and are clearly stated since they are key elements of the Risk Assessment that was peer reviewed.

It is also important to note that CSAS documents are not "evergreen" but that they reflect the agreed to opinions and evidence assembled on the day of the meeting. And this is clearly communicated to Management when providing them with the formal advice following our peer-review meetings. And we did flag in the SAR a recommendation that management should remain adaptive to the emergence of new information. And DFO does strive to keep Management

informed of new scientific developments as they emerge thus allowing Management to adjust their approaches as required.

Please don't hesitate to contact me if you have any further questions.

Thank you,  
Gilles  
Co-chair

**From:** [REDACTED]  
**Sent:** Sunday, March 17, 2019 8:44 AM  
**To:** Weber, Lily <Lily.Weber@dfo-mpo.gc.ca>; Burgetz, Ingrid <Ingrid.Burgetz@dfo-mpo.gc.ca>; Waddington, Zac <Zac.Waddington@dfo-mpo.gc.ca>; Struthers, Alistair <Alistair.Struthers@dfo-mpo.gc.ca>; Gagne, Nellie <Nellie.Gagne@dfo-mpo.gc.ca>; 'Nathalie.N.Bruneau@inspection.gc.ca' <Nathalie.N.Bruneau@inspection.gc.ca>; 'Myron.Roth@gov.bc.ca' <Myron.Roth@gov.bc.ca>; [REDACTED] Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; [REDACTED] 'espen.rimstad@nmbu.no' <espen.rimstad@nmbu.no>; 'niven@vet.dtu.dk' <niven@vet.dtu.dk>; 'mark.powell@hi.no' <mark.powell@hi.no>; 'iagardner@upei.ca' <iagardner@upei.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Polinski, Mark <Mark.Polinski@dfo-mpo.gc.ca>; Mimeault, Caroline <Caroline.Mimeault@dfo-mpo.gc.ca>; Holt, Kendra <Kendra.Holt@dfo-mpo.gc.ca>; Johnson, Stewart <Stewart.Johnson@dfo-mpo.gc.ca>; Jones, Simon <Simon.Jones@dfo-mpo.gc.ca>; [REDACTED]; 'tony.farrell@ubc.ca' <tony.farrell@ubc.ca>; [REDACTED] 'Gary.Marty@gov.bc.ca' <Gary.Marty@gov.bc.ca>; [REDACTED]  
Boily, France <France.Boily@dfo-mpo.gc.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>  
**Cc:** Olivier, Gilles <Gilles.Olivier@dfo-mpo.gc.ca>; Craig Stephen <cstephen@cwahc-rccsf.ca>; Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>  
**Subject:** Re: Draft PRV SAR For Your Review

Something has been nagging at me for some time now about this risk assessment and I feel I need to express my thoughts on this before this document and the process is finalized.

Sorry for the late comment on this.

But one of the key assumptions made in the risk assessment is:

At line 316 SAR - "Assuming that results from laboratory studies on the impact of PRV-1 infection in juvenile Sockeye Salmon are indicative of what occurs in the marine environment, it was concluded with reasonable certainty that the potential magnitude of consequences to Fraser River Sockeye Salmon abundance and diversity would be negligible."

And I am reminded of other CSAS reviews and scientific assessments (including through the Aquaculture Stewardship Council criterion reviews) looking at the effects of pesticides and herbicides on wild animal (fish) populations (such as deltamethrin and Alphamax and SLICE) during which the point is often raised that in laboratory studies it is shown that these chemicals are toxic to marine organisms and as such we should proceed cautiously with how we use them. And the typical response is "We can't do that because those findings are based on laboratory studies and we really have no way of determining whether those chemicals

will behave similarly or cause toxicity in the same manner in the real environment. There are too many unknowns".

This is a common refrain when dealing with chemical pollution issues.

Yet, for this assessment, despite all of the uncertainties identified in the process, we make just that kind of assumption.

Bear in mind that the laboratory conditions for the PRV challenge studies are nowhere near what happens in the real world. The water used in these studies is temperature controlled, sand-filtered and filtered with UV light to sterilize it. Flow through rates are modulated. There are no possibilities of co-infection. And the type and amount of feed being fed to the test organisms is not representative of what is found in nature. Neither is the key stressor (hand-swirling in a bucket).

I just wanted to point out this out and ask why can we or should we tolerate this assumption?

It is a key assumption and IMO, one that cannot and should not be made.

I may be out of line on bringing this forward but I cannot with good conscience not say anything about it.

Regards

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**From:** Weber, Lily <[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca)>

**Sent:** Thursday, March 7, 2019 5:42:30 AM

**To:** Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair; Gagne, Nellie; 'Nathalie.N.Bruneau@inspection.gc.ca'; 'Myron.Roth@gov.bc.ca'; [REDACTED] Miller-Saunders, Kristi; [REDACTED] 'espen.rimstad@nmbu.no'; 'niven@vet.dtu.dk'; 'mark.powell@hi.no'; 'iagardner@upei.ca'; Garver, Kyle; Polinski, Mark; Weber, Lily; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED] 'tony.farrell@ubc.ca'; [REDACTED] 'Gary.Marty@gov.bc.ca'; [REDACTED] Boily, France; Garver, Kyle

**Cc:** Olivier, Gilles; Craig Stephen; Parsons, Jay

**Subject:** Draft PRV SAR For Your Review

Dear Participants of the CSAS peer review on the risk to Fraser River Sockeye Salmon for PRV from Atlantic salmon farms in the Discover Islands area:

On behalf of the co-chairs (Gilles Olivier and Craig Stephen), attached please find the draft version of the Science Advisory Report (SAR) for the PRV risk assessment that we reviewed in January 2019.

We are seeking your comments and approval of this document. Recall that at the meeting we had agreed to the summary bullets, Recommendations and Other Considerations, so please focus your review on the other parts of the

report including the Introduction, Analysis, Sources of Uncertainty, Conclusions, etc. to assess if there are any factual errors or omissions or other comments. However, you will note that there are a few changes to the summary bullets, recommendations and other considerations which were made to improve clarity and grammar. Please let us know if you agree with these changes.

We would appreciate receiving your comments by **Friday, March 22, 2019**. After which we will review all input, finalise the report, seek the chairs' and internal approvals, and format for posting on the DFO web site.

Thank you.

**Lily Weber**

Science Advisor, Aquaculture, Biotechnology and Aquatic Animal Health Science Branch

Fisheries and Oceans Canada / Government of Canada

[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca) / Tel: [REDACTED]

Conseillère scientifique, Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques

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du Canada

Canada

s.16(2)(c)

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** March-31-19 9:22 AM  
**To:** [REDACTED]  
**Subject:** RE: Re:

Sorry, I forgot to get back to you. Yes I am around this week. My office phone is 250-756-7155. You can call Monday afternoon.

Kristi

---

**From:** [REDACTED]  
**Sent:** March 31, 2019 8:13 AM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re:

Hey Kristi,

I hope you're doing well. I was wondering if you'd be free to speak before Friday? I can also email you questions if that works better for you.

On Fri, Mar 29, 2019 at 11:38 AM [REDACTED] wrote:  
[REDACTED] wrote:  
Sounds good, Kristi.

I'm happy to have an informal, off-the-record conversation before doing any interview, so that you can learn as much about the story as you need to.

I do have ATIP documents.

[REDACTED]

On Fri, Mar 29, 2019 at 1:19 AM Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca<mailto:Kristi.Saunders@dfo-mpo.gc.ca>> wrote:

Hello [REDACTED]  
I am out of town until next week, but may try to give you a call tomorrow to find out more about your story. I assume you have ATIP documents if you are focusing on the 2015 CSAS on PRV.

Talk to you soon,  
Kristi

---

**From:** [REDACTED]  
**Sent:** March 25, 2019 9:10 PM  
**To:** Miller-Saunders, Kristi  
**Subject:**

s.19(1)

Hello Ms. Miller,



My name is [REDACTED] I am a journalist working on a story about the disagreements that took place between DFO scientists in 2015 during the drafting of the CSAS pertaining to PRV. I will likely be pitching this story to the National Observer, Hakai, or the Narwhal.

Would you be available for an interview sometime later this week or sometime next week? You can call me anytime at [REDACTED] if you would like to speak to me first.

Here is a sample of one of my most recent stories:

[REDACTED]

Thanks,

[REDACTED]

s.19(1)

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** April-02-19 3:49 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** FW: CSAS  
**Attachments:** Working paper 2 - PRV risk assessmentKM.pdf; Working Paper 1 - PRV Characterization\_KM.pdf

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

---

**From:** Miller-Saunders, Kristi  
**Sent:** January-22-19 11:12 PM  
**To:** DiCicco, Emiliano <Emiliano.DiCicco@dfo-mpo.gc.ca>  
**Subject:** CSAS

Here are the documents with my notes thus far. Need to organize them and put together a cohesive argument.

**Pages 231 to / à 282  
are duplicates of  
sont des duplicatas des  
pages 60 to / à 111**

**Pages 283 to / à 321  
are duplicates of  
sont des duplicatas des  
pages 21 to / à 59**

## Miller-Saunders, Kristi

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**From:** Holmes, John  
**Sent:** April-02-19 10:34 PM  
**To:** MacDougall, Lesley; Klaver, March; Kreiberg, Henrik; Jones, Simon; Miller-Saunders, Kristi; Withler, Ruth; Beacham, Terry; Higgins, Mark; Garver, Kyle; Johnson, Stewart; Houston, Kim; Chamberlain, Jon; Bianucci, Laura; Kennedy, Eddy; Webb, Allison; Salomi, Corino; MacWilliams, Christine; Sutherland, Ben; Coyle, Theraesa  
**Subject:** RE: ADM\_meeting\_2Apr19.ppt  
**Attachments:** ADM\_meeting\_2Apr19.ppt

STAR contribution in the attached.

Thanks Lesley

John

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**John Holmes**  
250-756-7145

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**From:** MacDougall, Lesley  
**Sent:** Tuesday, April 02, 2019 5:30 PM  
**To:** Klaver, March; Kreiberg, Henrik; Jones, Simon; Miller-Saunders, Kristi; Withler, Ruth; Beacham, Terry; Higgins, Mark; Garver, Kyle; Johnson, Stewart; Houston, Kim; Chamberlain, Jon; Bianucci, Laura; Kennedy, Eddy; Holmes, John; Webb, Allison; Salomi, Corino; MacWilliams, Christine; Sutherland, Ben; Coyle, Theraesa  
**Subject:** ADM\_meeting\_2Apr19.ppt

Hello all

Here is the draft presentation for the meeting with ADM science, Aquaculture Mgt Division, SEP, and Pacific region science staff for Thursday morning. If you have not already provided the input for your division / sector, please add to this file.

An Agenda and completed powerpoint presentation will be circulated tomorrow. Roughly:

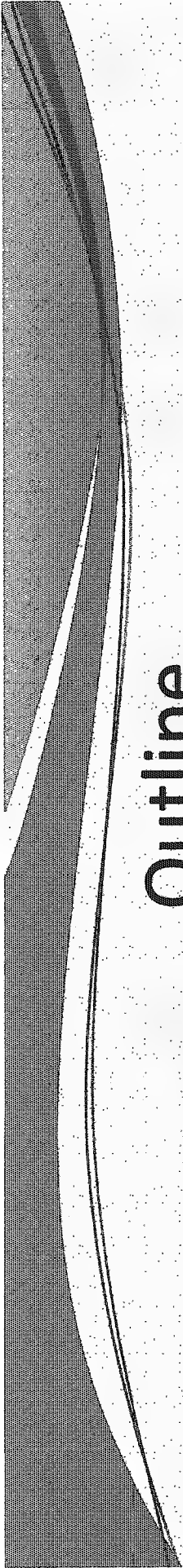
- 10 min – quick introduction
- 20 min each for SEP and AMD – priorities, areas needing science support, areas of overlap, new concerns? (this is shorter than what I originally requested – I'm hoping that this will allow more discussion time at the end)
- 10 min for each science division – highlights, areas that are supporting SEP and AMD

This leaves about 1 hour for discussion. I've proposed a few questions but am open to others suggesting questions:

1. How can we improve collaboration on research questions? – mechanisms for developing joint priorities
  - a. joint workshop
  - b. what about questions that are of interest to externals (e.g. First Nations)
  - c. what about other sectors in DFO
2. Can we identify areas with the most overlap potential? Are they consistent with priorities?
3. Are there areas where we can pool resources?
4. How is information from competitive funded research projects being applied?
5. How does information from primary publications get used in decision making?
  - a. Are better linkages needed between information in primary publications and management decision making

# Aquaculture, Salmon Enhancement Program, and Science: needs, research, priorities

April 4, 2019



# Outline

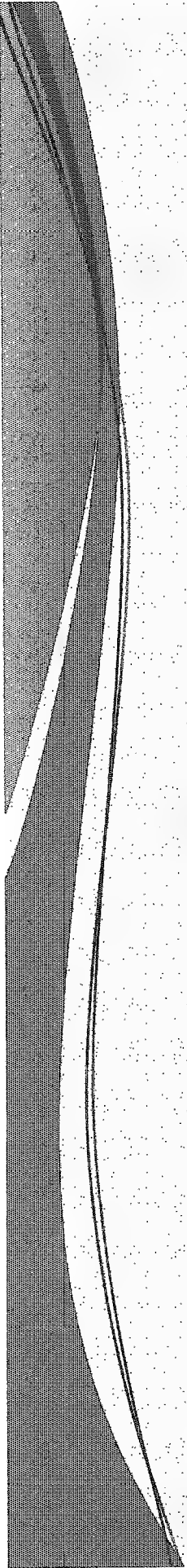
- AMD – current needs and priorities
- SEP – current needs and priorities
- SEP and Aquaculture Science Support: Overview
- Division overviews
  - ADGT
  - ESD
  - OSD
  - StAR
- Competitive-funded projects in Pacific science – research relevant to Aquaculture and SEP
- Discussion: SEP / Aquaculture priorities that overlap with current research, gaps, challenges



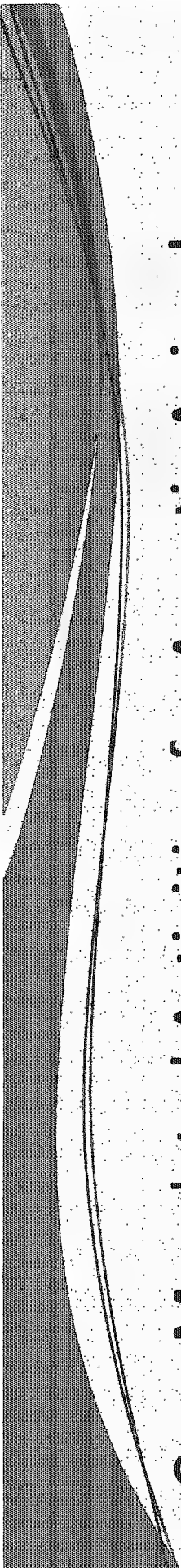
# SEP and Aquaculture Science Support: Overview

- **Ecosystem Science Division**
  - Near field effects program
  - Far field effects program
  - Ecosystem and long-term research on aquaculture impacts
- **Ocean Science Division**
  - Ocean Science Division
  - Coastal Oceanographic modeling for risk assessments and siting
  - Acoustic studies of fish migration to assess migratory overlap with aquaculture facilities
  - Profiling the distribution of HAB forming species
- **Aquatic Diagnostics Genomics and Technology**
  - Molecular Genetics: stock identification, parentage based tagging, genomics, eDNA, pathogen identification
  - Aquatic Animal Health: CFIA diagnostic support, histology, marine parasitology molecular virology and biology
  - Applied Technology: veterinary expertise, ROV and acoustic support, animal care, aquarium services, sclerochronology
- **Stock Assessment and Research Division**
  - Stock assessment, stock status





# Aquatic Diagnostics, Genomics and Technology (ADGT)



## Core Mandated Activities for Aquatic Animal Health in the Pacific Region

- Regulatory Testing (NAAHP) and Research (CAAHRD, PARR)
- Directed Research on aquatic animal pathogens (includes: Virology Program, Marine Parasitology Program, Wild/Farm Interactions, Strategic Salmon Health Initiative, Shellfish Health Research)
- Fish Pathology Program (Disease investigation and pathogen screening (BKD) for SEP hatcheries, Veterinary support, I&T disease screening)
- Response to Wild Fish Kills (DFO program and NGO support)
- Public Submissions



# Key Programs – Fish Health

- **Marine Virology**
  - Viral diagnostics and characterization - for the National Animal Health Program (NAAHP) for SEP, Fisheries, First Nations and Freshwater Fisheries Society of BC.
  - Viral epidemiology and disease ecology, interaction of wild and farmed fish
- **Marine Parasitology**
  - Structural and genomic characterization of parasites
  - Research into defense responses of the host to better understand resistance to parasite infection.
  - Development and application of treatment and immunisation strategies that enhance resistance within a population through the use of novel therapeutic, vaccine or immunostimulant formulations is studied.
- **Invertebrate Fish Health**
  - Addressing management-relevant knowledge gaps relating to invasive species, aquatic animal health, and biodiversity
  - Development of new regulatory tools (e.g. shellfish disease diagnostic test methods, validated molecular tool for environmental DNA based biomonitoring), Standard Operating Procedures, Test Method Protocols
  - Client services: molecular diagnostic testing and scientific expertise/advice for regulated shellfish disease.
  - Outputs: regulatory test results (NAAHP), advice within DFO-NAAHLS and to CFIA
- **National Aquatic Animal Health Program (NAAHP)**
  - co-delivered with the Canadian Food Inspection Agency (CFIA), the lead regulatory and administrative authority for NAAHP. DFO provides diagnostic testing, research, and scientific advice to support the program.
  - Disease Surveillance, Mortality and Disease Investigations, Reference Laboratory, Research Projects



# Key Programs– Applied Technology

- **Fish-ageing**
  - Inputs to stock assessment and fisheries management advice (supports programs in Aquatic Species Assessment and Research Division, Ecosystem Science Division), R&D in fish-ageing/climatology methods.
- **Aquarium Services**
  - Veterinary support, animal care oversight, services to Aquatic Animal Health/Molecular Genetics research for fish health and other experimental studies.
- **Fisheries acoustics and ROVs**
  - Inputs to stock assessment (e.g. Hake) and fisheries management advice
  - inputs to conservation and environmental protection (supports programs in Ecosystem Science Division e.g. marine spatial planning; supports Wild Salmon Policy implementation – tracking juvenile salmon outmigration).



# Key Programs - Molecular Genetics

- **Genomics/Salmon Health**
  - Genomic assessment of salmonid health and condition for wild, enhanced and farmed salmon
  - Molecular monitoring (e.g. diet assessments, plankton, microbes, environmental or eDNA)
  - Wild/cultured fish interaction risk assessment of novel organisms and their pathogens
- **Genetic Stock ID and population structure**
  - Genetic identification of samples on both a rapid (daily or intraweek) and regular (annual) basis on for domestic and international fisheries management and commitments under the PST and NPAFC
  - Genetic identification of organisms at the species, stock and individual level in partnered research applications
  - Genome-level genotyping for Parental based tagging (PBT) and other applications
- **Population Genetics: Aquaculture/forensics/enhancement**
  - Conservation measures and tools for monitoring of salmon management /conservation units and threatened populations, captive breeding programs
  - Genetic analysis for SEP Broodstock development, management and monitoring
  - Genetic analysis for sustainable development of aquaculture strains, tools for monitoring genetic wild/cultured interactions
- **Risk assessment of novel aquatic organisms**
  - Risk assessment methodology with a focus on identifying sources of uncertainty associated with lab-based empirical evaluations of genetically engineered fishes.
  - Genetic analysis of phenotypic changes associated with selective breeding and domestication of aquaculture strains of salmon. Genetic and fitness consequences of introgression of domesticated and wild-type genomes.
  - Analysis of adaptability of hatchery populations (heritability assessments, genomic evaluation of genetic contributions) across multiple generations.





## Spotlight: Supporting Sustainable Aquaculture

- Reinvestment in science: National Aquatic Animal Health Lab
  - Objective: increase output to diagnostic testing to support Canadian Food Inspection Agency's needs by 50% nationwide
  - Resources committed: one Eg-4 plus 320 K in lab renovations to increase diagnostic capacity
  - Completed 2018, and currently in use!
- Canadian Regulatory System for Biotechnology Investment: Salmon Genome Project
  - Objective: sequence all the genomes for Pacific salmon species
  - Resources committed: \$650K over 3 years
  - Timeline for delivery: end of 2019-20.
  - Progress to date: Coho Salmon genome complete, Chinook Salmon genome submitted to genebank; Sockeye Salmon genome sequencing underway; Pink Salmon and Chum Salmon sequencing underway; developing collaboration in Japan to do Amago and Masu salmon.
- Strategic Salmon Health Initiative
  - ~15,000 juvenile sockeye, Chinook and coho salmon assessed for >50 infectious agents.
  - Epidemiological analyses underway to identify associations between infection profiles and year-class strength and physiological indicators of disease.
  - Potential transmission dynamics between cultured and wild fish are also being assessed.
  - 9 novel viruses have been identified



# Spotlight: SEP support

- **EPIC4 Research:**

- *Exceeding sampling goals for hatcheries, baseline populations, and markers, and for genotyping. Published an important paper (Beacham et al. 2018. Evolutionary Applications), providing evidence that PBT is likely to replace CWT as a principal tool for managing hatchery supplementation.*

- **PBT and GSI:**

- Enables in-season CU-based catch estimation (and estimation of exploitation rate when escapements are monitored) for all Canadian CUs
- Enables identification of stray hatchery fish in natural populations and evidence of successful spawning leading to introgression
- Enables management of conservation hatcheries to limit domestication and evaluate success of spawning in natural environment

- **CSAS Advice:**

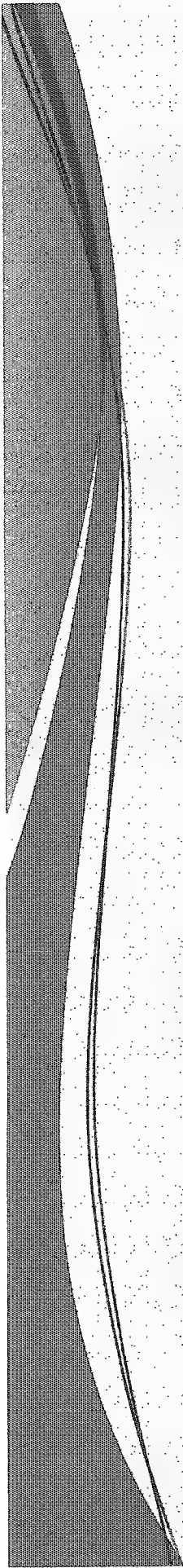
- Review of genetically based targets for enhanced contributions of Chinook (September 2017)
- Magnitude of Straying (anticipated Summer 2019)



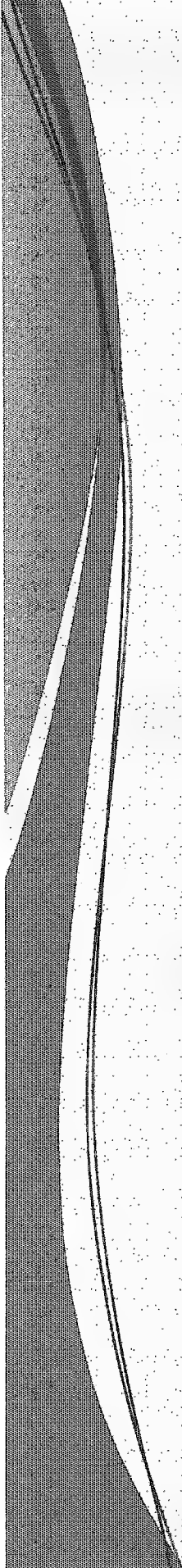
# CSAS – risk assessment frameworks

- Assessment of the risk to Fraser River Sockeye Salmon due to Infectious Hematopoietic Necrosis Virus (IHNV) transfer from Atlantic Salmon farms in the Discovery Islands, British Columbia.
- Risk Assessment for the bacterial pathogens *Aeromonas salmonicida*, *Renibacteria salmoninarum*, *Yersinia ruckeri* and *Piscirickettsia salmonis*, peer review completed.
- Risk Assessment for PRV transfer from Atlantic salmon farms to Fraser River Sockeye Salmon - peer review meeting held January 28-30, 2019.





# Ecosystem Sciences Division (ESD)



# Ocean Sciences Division (OSD)



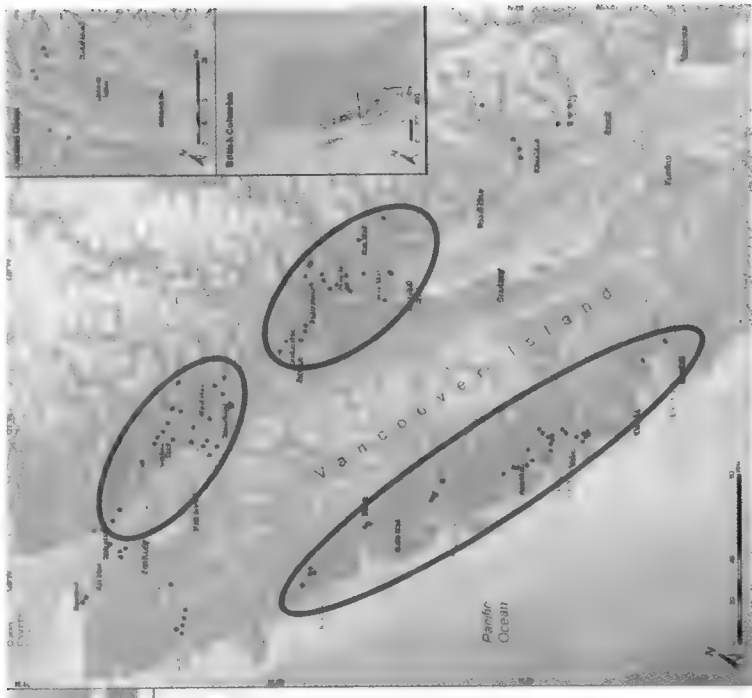
## Aquaculture Science within Ocean Sciences Division

### Current/recent activities

- Development of coastal oceanographic models for science advice supporting decision making/policy enhancements on:
  - Sea lice transfer and risk of farm-to-farm transmission (BCSF)
  - Pathogen transfer risk assessment and siting guidelines (Cohen)
  - Framework for Aquaculture Risk Management including incorporation of a Precautionary Approach (DFO Dec 2018)
- Approaches for Area Based Management of aquaculture industry (DFO Dec 2018)
- Acoustic studies of fish migration to assess migratory overlap with aquaculture facilities
  - Inform pathogen transfer risk assessment (Cohen)
- Profiling the distribution of HAB forming species including those posing risk to aquaculture facilities

# OSD – Coastal Oceanographic Models

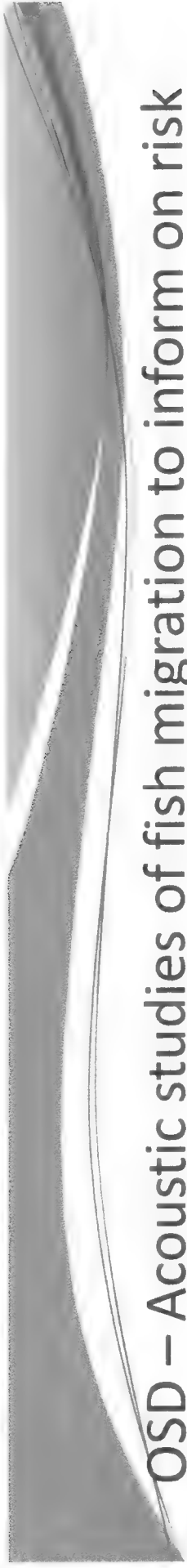
- OSD have been leading the development and application of coastal oceanographic models to inform on aquaculture issues for >15 years
- Model grids have been developed that encompass >80% of BC marine finfish aquaculture facilities



- Model outputs improve understanding of potential interactions between farms and natural environment by simulating the fate of both passive and biologically active particles.
- CSAS advice on pathogen transfer risk assessments and inform siting decisions

Culture permit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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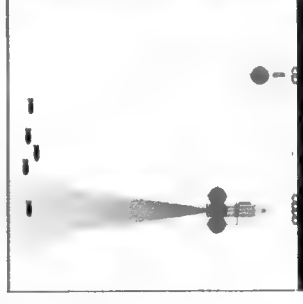
Farm to farm connectivity matrix



## OSD – Acoustic studies of fish migration to inform on risk

### Monitoring of wild juvenile salmon migration through the Discovery Passage area, in a channel that contains Atlantic salmon farms

Provides information on the large-scale migration timing and variability of wild juvenile salmon, as well as offering a measure of population interactions in vicinity of an aquaculture site (how long and how often are the fish in the area).



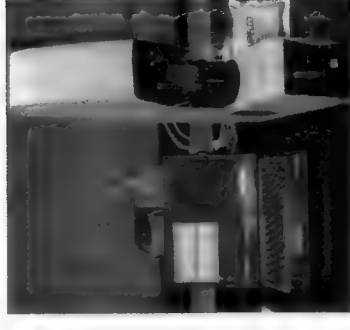
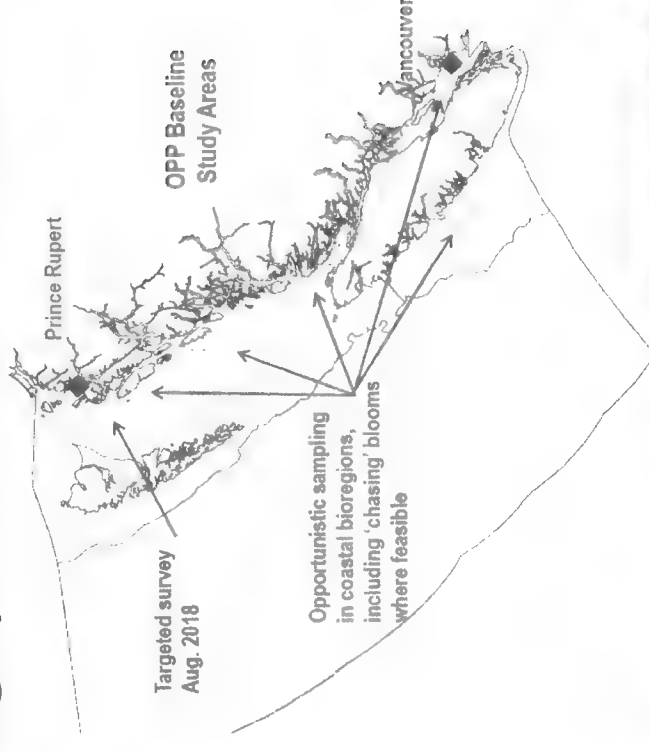
### Continuous monitoring of activity of wild fish in the direct vicinity of aquaculture sites using high resolution imaging sonar (DIDSON) mounted on aquaculture facilities

This provides estimates of close and direct contact (within a few meters) of wild Pacific juvenile salmon interactions with aquaculture infrastructures.

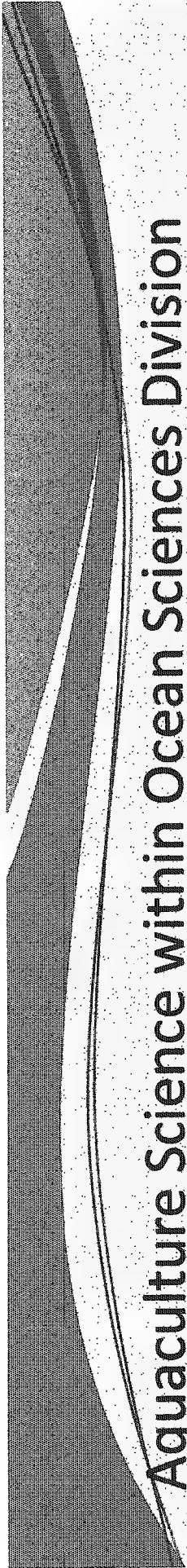


## OSD – Harmful Algal Bloom forming species and biotoxins

- Investigate distribution and abundance of HAB-forming species in coastal BC waters and sediments.
- Develop and apply methods to identify and quantify algal biotoxins in seawater and sediments.
- Document the distribution, concentration and persistence of these biotoxins in the waters and sediments of coastal BC.
- Examine associations among HAB-forming species, biotoxins and environmental conditions in BC.



Liquid chromatography-tandem mass spectroscopy (LC-MS/MS) equipment<sup>17</sup>

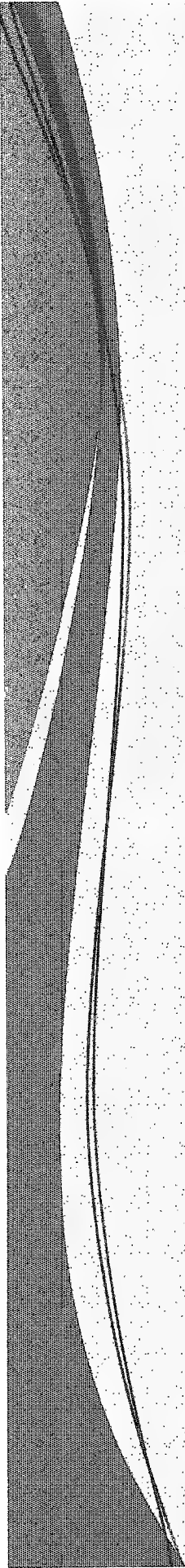


## Aquaculture Science within Ocean Sciences Division

### Future Opportunities

- Expand application of models to inform siting guidelines/decisions to establishment of discrete coastal zones for area-based management.
- Advancement of bio-geochemical models to improve understanding of development of low DO zones and HABs
- Leverage recent advances in modelling capabilities developed under OPP to establish operational coastal oceanographic models for aquaculture producing regions (real time forecasts)
- Examine data stream from new satellite ocean colour sensors to monitor long terms change in near surface water quality around aquaculture facilities.





# Stock Assessment and Research Division (StAR)





# Stock Assessment

- Fishery Independent Survey Activities
  - Groundfish – Synoptic bottom trawl (N&S), IPHC setline surveys, inshore hardbottom setline
  - Pelagics - herring in 5 major areas, sardine
  - Marine invertebrates – dive surveys (urchins, sea cucumbers, geoduck, Northern Abalone), shrimp trawl
  - Salmon – high seas & SOG (ESD), terminal area escapement + catch monitoring
- Data Management
  - Abundance index data
  - Biological data
  - Age data
  - Data requests – internal and external



# Stock Assessment

- Advice
  - Stock Status (catch, index, biological data)
  - Impact of Future Harvest on Stock (projections with different harvest scenarios)
  - Management Procedure Performance
- Analysis to Support Advice to Clients
- Models:
  - Simple trends with a lower fishery cutoff,
  - Surplus production models (catch only),
  - Statistical catch-at-age
  - Bayesian statistical catch-at-age (state of the art)



# Stock Assessment

- Management Strategy Evaluation – simulation testing of management procedures using fishery objectives developed by clients/stakeholders to measure performance; more collaborative way to work, requires more input from clients that they are used to providing
- Iterative process of refinement
- Requires high technical modelling capacity
- Current MSE processes: Herring, Sablefish, Pacific Hake, Albacore Tuna, Sockeye and Chinook Salmon
- Pacific Region scientists well placed to lead nationally (Bill C-68)



# Stock Assessment

- Data limited tools – develop advice on stocks for which time series don't exist (or are not long) and/or available data are incomplete, i.e., data are not sufficient to compile a statistical catch-at-age model
- Developed through partnership with UBC (T. Carruthers) and DFO (Robyn Forrest)
- Applied to Groundfish species, beginning work on marine invertebrates, looking at adaptations for short-lived species and salmon
- Important development for Pacific Region because of the number of data-limited stocks (Bill C-68)



# Stock Assessment - Salmon

- Support AMD and SEP
- WSP Integrated Biological Status Assessments (Fraser Sockeye, Southern BC Chinook, Interior Fraser coho); data-intensive exercise
- Outlook for Pre-season planning document (aggregate of CUs)
- Fraser sockeye pre-season forecast (CUs & MUs)
- Pre-season and post-season assessments of stock status under PST (abundance category and exploitation rate)
- State of wild salmon (aggregate of CUs) passing aquaculture facilities
- SEP – integrated planning process (rebuilding vs. stock assessment)



# Questions:

How can we improve collaboration on research questions? – mechanisms for developing joint priorities

- joint workshop
- what about questions that are of interest to externals (e.g. First Nations)
- what about other sectors in DFO

Can we identify areas with the most overlap potential?

Are they consistent with priorities?

Are there areas where we can pool resources?





# Questions:

How is information from competitive funded research projects being applied?

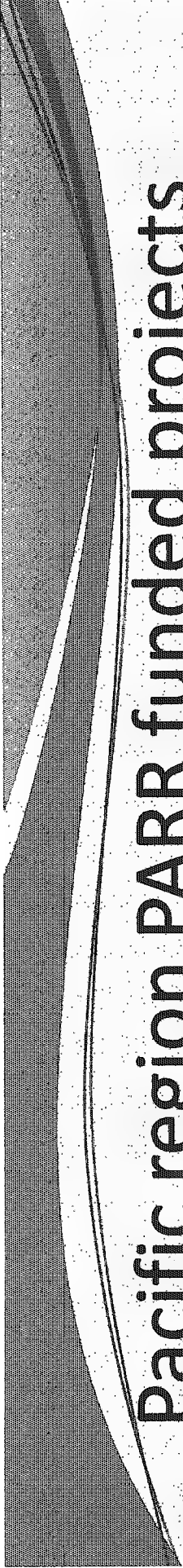
How does information from primary publications get used in decision making?

- Are better linkages needed between information in primary publications and management decision making

# Additional info

Questions posed by Broughton First Nations	DFO Science – current work, proposed work	DFO Aquaculture Management requests/priorities	Independent Expert Panel on Aquaculture Science Recommendations (Dr. Nemer)	Province of BC recommendations
<p>What is the "pathogen baseline" of wild stocks?</p> <ul style="list-style-type: none"> <li>○ Presence/absence of disease and disease agents.</li> <li>○ Pathogen infection rate/duration of 'clean' fish once introduced to farms</li> <li>○ At what point does a population become a 'source' of infection?</li> </ul>	<p>Investigations underway to understand: What are the pathogens found in wild salmon? (Kristi Miller-Saunders)</p> <p>Potential transmission dynamics between cultured and wild fish</p> <p>Identify associations between infection profiles and wild salmon year-class strength and indicators of disease. (Kristi Miller-Saunders)</p> <p>Specific to PRV: (Kyle Garver)</p> <ul style="list-style-type: none"> <li>• When do farms become PRV positive?</li> <li>• How are they becoming PRV infected?</li> <li>• Are infected farms contagious and how much PRV do they shed?</li> <li>• How much PRV is required to initiate an infection?</li> </ul> <p>Investigations into other pests and pathogens is also continuing, including sea lice, amoebic gill disease, Piscirickettsia salmonis (Simon Jones, Stewart Johnson).</p>	<p>Risk Assessments with respect to highest risk pathogens and transfer of disease between cultured and wild fish stocks in response to Cohen Commission recommendations as well as PRV risk assessment.</p>	<p>Recommendation 1: Implement best practices, synthesize available scientific evidence on aquaculture risks</p>	
<p>How do First Nations environmental / ecological/ traditional knowledge get incorporated appropriately?</p>	<p>Engagement and partnerships – working on a 5 year science strategic plan</p> <p>First Nations employment and training in lab and field.</p> <p>Proposed DFO/UBC education partnership for aquaculture master's certificate program – PSEC.</p>	<p>Engagement and partnerships – working on a 5 year science strategic plan and will be consulting with FNs through forum such as the FNFC's ACC</p> <p>Area based management – opportunities for First Nations monitoring programs on an area specific basis</p> <p>Involvement of FN vet on the Fish Health Advisory Committee</p>	<p>Recommendation 1: Incorporate Indigenous and local knowledge in decision making</p> <p>Recommendation 5: DFO should develop a clear overarching scientific vision and a corresponding multi-year research plan</p>	<p>Recommendation 3: Increased capacity for First Nations monitoring and salmon restoration</p> <p>Provide meaningful employment for the First Nations community members</p>





## Pacific region PARR funded projects 2017-18 (projects underway 2018-19)

- eDNA biosurveillance – shellfish aquaculture movements
- Biomonitoring – impacts of salmon aquaculture on marine benthic communities
- Marine reservoirs of infectious agents associated with proliferative gill disorders
- Investigation of PRV in development of disease
- VHSV evolution and adaptation
- Characteristics of BC isolates of *Piscirickettsia salmonis*
- Advanced developments in FVCOM for Discovery and Broughton



## **Pacific region ACRDP projects (underway 2018-19)**

- **Prevalence and transmission dynamics of PRV in marine environment**
- **Pile perch as a cleaner for sea lice**
- **Occurrence, distribution and causes of gill disease**
- **Visceral mycoses in Atlantic salmon and the role of fungal pathogens**
- **Acoustic monitoring of wild fish interactions with aquaculture sites – phase 2**



## Pacific region ACRDP projects (ongoing)

- FVCOM validation
- Seasonal mortality in Pacific oysters
- Improving fertilization success in Arctic Charr
- Development of diets and feeding strategies for the implementation of sea lice cleaner perch
- Epidemiology of net pen liver disease

Questions posed by Broughton First Nations	DFO Science – current work, proposed work	DFO Aquaculture Management requests/priorities	Independent Expert Panel on Aquaculture Science Recommendations (Dr. Nemer)	Province of BC recommendations
Density of farms, location – zone of influence	<p>Advanced developments in FVCOM for Discovery and Broughton (Laura Blantucci) – will also have applications to Area Based Management (these advancements are ongoing – project funded for 2019-20)</p> <p>Biomonitoring – impacts of salmon aquaculture on marine benthic communities (2018 PARR funded Cathryn Abbott)</p> <p>Marine reservoirs of infectious agents associated with proliferative gill disorders (Simon Jones) – PARR funded for 2019-20</p> <p>Prevalence and transmission dynamics of PRV in marine environment (Kyle Garver ACRDP)</p> <p>Acoustic monitoring of wild fish interactions with aquaculture sites – phase 2 (Stephan Gauthier ACRDP)</p>	<p>Evaluation of potential metrics / indicators for area based management of connected Fish Farms (2017AQU02)</p> <p>Evaluation of fallowing as a strategy for disease mitigation in marine finfish growout facilities (2017AQU03)</p> <p>Finfish aquaculture sea lice management in British Columbia (2012AQU05)</p> <p>Area based management could also lead to area specific thresholds and performance based measures</p>	<p>Recommendation 2: use best practices to characterize and understand the potential risks and impacts associated with aquaculture. – pathways of effects</p> <p>Recommendation 3: use quantitative methodologies and risk-science approaches to develop an Integrated Risk Management Framework (IRMF) that ensures that all relevant factors are properly considered in aquaculture decisions.</p> <p>Recommendation 1: best practices including systematic reviews / peer review</p>	
Farms – do they exacerbate climate change impacts?	<p>Links between HABs, biofouling, disease – will they be worse with climate change (early proposal stage for funding – Andrew Ross - ACRDP)</p> <p>Seasonal mortality in Pacific oysters (Chris Pearce – ACRDP early proposal phase)</p>	No requests at present and no expectation for AMD to make a request in this area given other priorities		

Questions posed by Broughton First Nations	DFO Science – current work, proposed work	DFO Aquaculture Management requests/priorities	Independent Expert Panel on Aquaculture Science Recommendations (Dr. Nemer)	Province of BC recommendations
Closed containment and lice treatment technologies	eDNA biosurveillance – shellfish aquaculture movements (Cathryn Abbott) Pile perch as a cleaner for sea lice (Stewart Johnson, ACRDP) Development of diets and feeding strategies for the implementation of sea lice cleaner perch (Ian Forster – Ongoing ACRDP funded project) Closed containment workshops last fall – held at PSEC, 2 reports were produced	AMD NHQ is leading the development of an alternative technology study and there is FN representative on the Steering Committee. This study will provide information to inform any future policy direction and/or management measures regarding closed containment and/or alternative technologies.  AMD has not requested information from Science on specific alternative treatment technologies, but is working on enhanced management of sea lice. See also above related to research questions on sea lice.		<b>Recommendation 6:</b> consider immediate incentives and opportunities to collaborate -- new farming techniques that reduce potential risk to wild salmon
Factors for risk-based decision making	CSAS peer review of risk assessment framework – risks to Sockeye first, Risk Assessment for the bacterial pathogens <i>Aeromonas salmonicida</i> , <i>Renibacterium salmoninarum</i> , <i>Yersinia ruckeri</i> and <i>Piscirickettsia salmonis</i> , PRV peer reviews completed.  moving to other spp in other areas -- input to what populations, spatial and temporal scales.	DFO has committed to developing a framework for risk based decision making as a response to the CESD Audit. This is being led by NHQ AMD. In the Region, we have a decision making framework, pathways of effects and implementation that can be provided to FN to explain how our current approach to aquaculture management considers risk.  AMD continues to employ adaptive management and is currently considering the results of the CSAS peer review on the various pathogens as part of its management regime.	<b>Recommendation 3:</b> use quantitative methods and risk-science approaches to develop an Integrated Risk Management Framework (IRMF) that ensures that all relevant factors are properly considered in aquaculture decisions.  <b>Recommendation 1:</b> best practices including systematic reviews / peer review	

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** April-03-19 8:59 AM  
**To:** Miller-Saunders, Kristi  
**Subject:** FW: papers as discussed  
**Attachments:** jfd\_12951(1).pdf; Zhang et al (in press).pdf; Polinski et al (in press).pdf; viruses-11-00112.pdf; viruses-11-00112.pdf

*Kristi Miller-Saunders, PhD*

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**From:** Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>

**Sent:** January-28-19 6:07 PM

**To:** Olivier, Gilles <Gilles.Olivier@dfo-mpo.gc.ca>; cstephen@cwahc-rcsf.ca; Burgetz, Ingrid <Ingrid.Burgetz@dfo-mpo.gc.ca>; Waddington, Zac <Zac.Waddington@dfo-mpo.gc.ca>; Struthers, Alistair <Alistair.Struthers@dfo-mpo.gc.ca>; Gagne, Nellie <Nellie.Gagne@dfo-mpo.gc.ca>; Nathalie.N.Bruneau@inspection.gc.ca; Myron.Roth@gov.bc.ca; [REDACTED] Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; [REDACTED] espen.rimstad@nmbu.no; niven@vet.dtu.dk; mark.powell@hi.no; iagardner@upei.ca; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Polinski, Mark <Mark.Polinski@dfo-mpo.gc.ca>; Weber, Lily <Lily.Weber@dfo-mpo.gc.ca>; Mimeault, Caroline <Caroline.Mimeault@dfo-mpo.gc.ca>; Holt, Kendra <Kendra.Holt@dfo-mpo.gc.ca>; Johnson, Stewart <Stewart.Johnson@dfo-mpo.gc.ca>; Jones, Simon <Simon.Jones@dfo-mpo.gc.ca>; [REDACTED] tony.farrell@ubc.ca; [REDACTED] Gary.Marty@gov.bc.ca; [REDACTED] Boily, France <France.Boily@dfo-mpo.gc.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>  
**Cc:** Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>  
**Subject:** papers as discussed

All,

Please see attached the in press papers as discussed to day and as requested.

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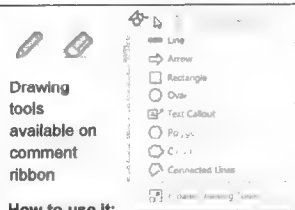
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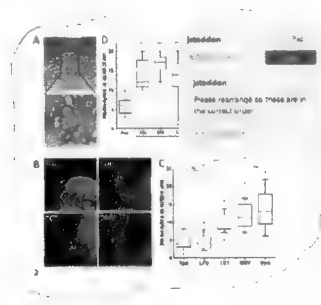


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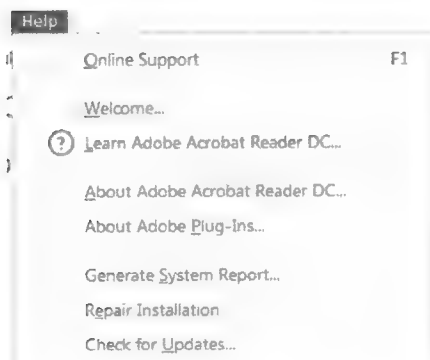
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ORIGINAL ARTICLE



# Infectious agent detections in archived Sockeye salmon (*Oncorhynchus nerka*) samples from British Columbia, Canada (1985–94)

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## Abstract

In response to concerns that novel infectious agents were introduced through the movement of eggs as Atlantic salmon aquaculture developed in British Columbia (BC), Canada, we estimated the prevalence of infectious agents in archived return-migrating Sockeye salmon, from before and during aquaculture expansion in BC (1985–94). Of 45 infectious agents assessed through molecular assays in 652 samples, 23 (7 bacterial, 2 viral and 12 parasitic) were detected in liver tissue from six regions in BC. Prevalence ranged from 0.005 to 0.83 and varied significantly by region and year. Agent diversity ranged from 0 to 12 per fish (median 4), with the lowest diversity observed in fish from the Transboundary and Central Coast regions. Agents known to be endemic in Sockeye salmon in BC, including *Flavobacterium psychrophilum*, Infectious haematopoietic necrosis virus, *Ceratonova shasta* and *Parvicapsula minibicornis*, were commonly observed. Others, such as *Kudoa thyrsites* and *Piscirickettsia salmonis*, were also detected. Surprisingly, infectious agents described only recently in BC salmon, *Ca. Branchiomonas cysticola*, *Parvicapsula pseudobranchicola* and *Paranucleospora theridion*, were also detected, indicating their potential presence prior to the expansion of the aquaculture industry. In general, our data suggest that agent distributions may not have substantially changed because of the salmon aquaculture industry.

## KEYWORDS

archived, BioMark, Fraser River, infectious agents, Skeena, Sockeye salmon

## 1 | INTRODUCTION

Pacific salmon are a crucial component of both the aquatic and terrestrial ecosystems of the Pacific Northwest (Cederholm, Kunze, Murota, & Sibatani, 1999; Gende, Edwards, Willson, & Wipfli, 2002). Sockeye salmon (*Oncorhynchus nerka*) is one of the most abundant Pacific salmon species in British Columbia (BC), Canada, with profound social, cultural, symbolic, recreational and economic importance to First Nations communities and west coast residents generally (Council, 1996; Lichatowich & Lichatowich, 2001). With a

life span ranging from four to six years, most Sockeye salmon populations spend the first one to two years in freshwater nursery lakes, followed by migration to marine waters and a return to their natal habitats to spawn after two or three years (Burgner, 1991).

Sockeye salmon have genetically and geographically distinct populations, acquired through their evolutionary history and their strong homing fidelity. They spawn in the particular lake or stream in which they were born, in a particular season, and do not interbreed with fish from other stocks (Ricker, 1972). The largest Sockeye salmon stocks in Canada belong to the Fraser, Skeena, Nass and

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## High-load reovirus infections do not imply physiological impairment in salmon

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12 <sup>†</sup> These authors have contributed equally to this work

13 **Keywords: piscine orthoreovirus, salmon, aerobic performance, heart inflammation, viremia,**  
14 **nucleated erythrocytes.**

### 15 **Abstract**

16 The recent ubiquitous detection of PRV among salmonids has sparked international concern about  
17 the cardiorespiratory performance of infected wild and farmed salmon. Piscine orthoreovirus (PRV)  
18 has been shown to create substantial viremia in salmon by targeting erythrocytes for principle  
19 replication. In some instances, infections develop into heart and skeletal muscle inflammation  
20 (HSMI) or other pathological conditions affecting the respiratory system. Critical to assessing the  
21 seriousness of PRV infections are controlled infection studies that measure physiological impairment  
22 to critical life support systems. Respiratory performance is such a system and here multiple indices  
23 were measured to test the hypothesis that a low-virulence strain of PRV from Pacific Canada  
24 compromises the cardiorespiratory capabilities of Atlantic salmon. Contrary to this hypothesis, the  
25 oxygen affinity and carrying capacity of erythrocytes was unaffected by PRV despite the presence of  
26 severe viremia, minor heart pathology and transient cellular activation of antiviral response  
27 pathways. Similarly, PRV-infected fish had neither sustained nor appreciable differences in  
28 respiratory capabilities compared with control fish. The lack of functional harm to salmon infected  
29 with PRV in this instance highlights that, in an era of unprecedented virus discovery, detection of  
30 viral infection does not necessarily imply bodily harm and that viral load is not always a suitable  
31 predictor of disease within a host organism.

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# Piscine orthoreovirus demonstrates high infectivity but low virulence in Atlantic salmon of Pacific Canada

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## ABSTRACT

Piscine orthoreovirus (PRV) is ubiquitous in farmed Atlantic salmon and sometimes associated with disease – most notably, Heart and Skeletal Muscle Inflammation (HSMI). However, PRV is also widespread in non-diseased fish, particularly in Pacific Canada, where few cases of severe heart inflammation have been documented. To better understand the mechanisms behind PRV-associated disease, this study investigated the infection dynamics of PRV from Pacific Canada and the potential for experimental passage of putatively associated heart inflammation in Pacific-adapted Mowi-McConnell Atlantic salmon. Regardless of the PRV source (fish with or without HSMI-like heart inflammation), infections led to high-load viremia that induced only minor focal heart inflammation without significant transcriptional induction of inflammatory cytokines. Repeated screening of PRV dsRNA/ssRNA along with histopathology and gene expression analysis of host blood and heart tissues identified three distinct phases of infection: (1) early systemic dissemination and replication without host recognition; (2) peak replication, erythrocyte inclusion body formation and load-dependent host recognition; (3) long-term, high-load viral persistence with limited replication or host recognition sometimes accompanied by minor heart inflammation. These findings contrast previous challenge trials with PRV from Norway that induced severe heart inflammation and indicate that strain and/or host specific factors are necessary to initiate PRV-associated disease.

## Introduction

Piscine orthoreovirus (PRV) infections of ocean-farmed salmon are widespread, and most farmed populations probably become infected at some point during a production cycle. These infections usually occur in the absence of overt disease; however, in some farming instances PRV has been associated with the development of serious disease syndromes. Specifically, a PRV subtype from Norway has been demonstrated to be the etiological agent of a disease known as Heart and Skeletal Muscle Inflammation (HSMI) of farmed Atlantic salmon (*Salmo salar*)<sup>1</sup>. This disease represents one of the most significant infectious diseases currently affecting Norwegian Atlantic salmon production<sup>2</sup>. Alternative PRV subtypes have also been identified in association with erythrocytic inclusion body syndrome (EIBS) in farmed Japanese Coho salmon (*Oncorhynchus kisutchi*)<sup>3</sup> as well as with an HSMI-like disease in farmed Norwegian rainbow trout (*Oncorhynchus mykiss*)<sup>4</sup>; both conditions have been associated with high morbidity/mortality in some situations.

In contrast, PRV is also prevalent in many asymptomatic farmed salmon, particularly along the Pacific coast of North America, which often have high viral loads without associated lesions<sup>5</sup>. Indeed, despite the presence of PRV in farmed Atlantic salmon of British Columbia, Canada, for more than 30 years with presumed high prevalence during much of that time<sup>5</sup>, heart and/or muscle inflammation has not had a significant impact to farm production<sup>6</sup>. Two studies have reported HSMI-like disease in Pacific Canada<sup>7,8</sup> (originally reported as HSMI but we say 'HSMI-like' as PRV etiology is uncertain). However, the condition is generally rare and not all fish diagnosed with these HSMI-like lesions were infected with PRV<sup>7</sup> – a circumstance that has also been described previously<sup>9</sup>. Further, clinical presentation of disease as originally used in the case definition of HSMI as it occurs in Norway<sup>10,11</sup> has never been reported in Pacific Canada.

A juxtaposition in virulence following PRV infection has also been highlighted in laboratory exposure trials. In Norway, exposure of Atlantic salmon to PRV by intra-peritoneal injection, cohabitation, and anal intubation have all resulted in moderate to severe heart lesions between 4-8 weeks post challenge<sup>1,12</sup>. In Canada, comparable PRV exposure trials using both intra-peritoneal injection and cohabitation have resulted in systemic PRV loads that were similar (if not greater) than those achieved in Norway, but no heart or muscle inflammation occurred during a similar time frame in either Atlantic or Sockeye (*Oncorhynchus nerka*) salmon<sup>13</sup>. Thus, it is currently unclear whether the HSMI-like lesions observed in Atlantic salmon of Pacific Canada are indeed HSMI; i. e., initiated primarily as a result of PRV.

One notable difference between Norwegian and Pacific Canada PRV exposure trials concerns the disease state of the donor fish from which PRV was sourced for exposure. Where Norwegian-based experiments have used PRV collected from Atlantic salmon with HSMI, Pacific Canada studies have used virus from non-diseased fish owing to the lack of regional availability for HSMI-diseased specimens. Thus, the previous inability of PRV from Pacific Canada to cause HSMI or any other disease symptoms in Atlantic salmon following challenge might be a result of genetic differences that have rendered at least some PRV variants of Pacific Canada less virulent. The fact that PRV sequenced from Pacific Canada to date appears phylogenetically distinct relative to PRV from Norway, which encompasses multiple amino-acid substations on various genomic segments, provides evidence in support of such a hypothesis<sup>14</sup>. However, the HSMI-like disease reported in PRV-infected Atlantic salmon from Pacific Canada supports an alternate hypothesis that at least one PRV variant from Pacific Canada may have caused severe heart inflammation in farmed Atlantic salmon of western North America<sup>7,8</sup>.

In this study, we compared PRV of Pacific Canada derived from two sources: 1) non-diseased fish and 2) fish with significant inflammation in the heart and skeletal muscle characteristic of HSMI. Our goal was to identify (i)



if differences in virulence could be explained by genetic differences in the virus, and (ii) if, like in Norwegian studies, HSML-like lesions as they has been recently diagnosed in Pacific Canada could be demonstrated as an infectious and transmissible disease within farmed Atlantic salmon. Further, we sought to improve upon the general understanding of PRV infection dynamics within farmed Atlantic salmon of Pacific Canada in the hope of identifying differences from Norway challenge trials that might help to explain the mechanisms for PRV virulence and pathogenesis.

## Results

**Preface concerning the diagnosis of HSML.** The original case definition of HSML in Norway was founded on a set of clinical disease, gross pathology, and histological characteristics that could be differentially diagnosed from other common transmissible muscular disorders (e.g., cardiomyopathy syndrome and pancreas disease) using histopathology<sup>10, 11</sup>. Currently, histopathology is still used to diagnose clinical HSML and also to differentially diagnose subclinical cases of HSML, cardiomyopathy syndrome (CMS) and pancreas disease (PD) in farmed Atlantic salmon of Norway<sup>15</sup>. Although these diagnoses are made solely upon histological evaluations, it is generally accepted that the primary agent responsible for each disease is a unique virus: PRV is the primary agent responsible for HSML<sup>1</sup>, piscine myocarditis virus (PMCV) is the primary agent responsible for CMS<sup>16</sup>, and salmon alpha virus (SAV) is the primary agent responsible for PD<sup>17</sup>. Indeed, although environmental and/or host contributing factors may explain the often exacerbated severity of HSML in a field relative to laboratory setting, PRV appears to be the sole infectious agent associated with the unique set of histopathological criteria that defines HSML in Norway<sup>1, 15, 18</sup> and to our knowledge HSML has never been used to classify a disease state in Norway where PRV has been confirmed to be absent.

Two recent studies from Pacific Canada have also used the term HSML to classify subclinical heart disease of farmed Atlantic salmon based on histopathology in accordance with their own definitions that are similar to those previously reported in Norway – namely, moderate to severe heart inflammation sometimes accompanied by skeletal muscle inflammation<sup>7, 8</sup>. Although the presumed commonality for the heart and skeletal muscle lesions in these Canadian studies relative to HSML diagnosed in Norway is the causation by PRV, there is far less evidence in Canada to support that PRV is indeed the key component for initiating this disease state. Particularly given that these modified definitions based on heart pathology alone have occasionally been observed in the absence of PRV<sup>5, 7</sup>. Consequently, if HSML diagnosis is based solely on histopathological heart lesions which can occur in the absence of PRV, then PRV cannot be assumed to be *the* causative agent of the disease, but rather one of multiple stand-alone or synergistic putative factors.

For this study, we reserve the designation of HSML in field environments to cases as defined by Wiik-Nielsen and colleagues<sup>15</sup> – presence of cellular epicarditis; moderate-to-severe inflammation and necrosis especially in the ventricle (inflammation predominant); inflammation of the red skeletal muscle a supportive finding – for which PRV is the likely primary etiologic agent. We use the term HSML-like for cases which fit the definition of HSML as presented above but have questionable etiology, and use the term idiopathic cardiopathy in all other instances of heart associated pathology for which PRV may or may not be a contributing cause. In a laboratory setting where external factors are controlled, we adopt the modified diagnosis of HSML previously used in assessing most experimental challenge trials in Norway; namely, histological visualization of moderate to severe heart

inflammation that may or may not be accompanied by skeletal muscle inflammation but is clearly associated with a PRV infection, e.g. absent from the control population<sup>1, 19, 20, 21, 22</sup>.

# **Case Report for HSMI-like disease in farmed Atlantic salmon of Pacific Canada.** Idiopathic

cardiopathy of a severity to cause morbidity or death (for which putative HSMI or HSMI-like lesions would be encompassed as a subset) is uncommon among British Columbia farmed Atlantic salmon and has historically been diagnosed in 1- 3% of surveillance samples taken from moribund or recently deceased fish on farm sites since the early 1990s<sup>23, 24, 25, 26</sup>. More recently, Fisheries and Oceans Canada Aquaculture Management Division conducts a regulatory Fish Health Auditing and Surveillance Program that from 2014-2017 sampled 2,960 moribund or recently deceased farmed Atlantic salmon for histopathology (<https://open.canada.ca>). These fish were collected during 407 audits of Atlantic salmon marine farms. Various types of idiopathic cardiopathy were diagnosed as a cause or marker of death in 62 (2.1%) of the sampled fish, and one of these fish (0.03% of 2,960) also had moderate skeletal muscle inflammation. Although some of the idiopathic cardiopathy observed in these samples (as well in samples from the early 1990s) might represent HSMI caused by PRV, a definitive diagnosis of HSMI is impossible to provide with any degree of certainty in these isolated occurrences.

However, on July 5<sup>th</sup>, 2016, microscopic lesions consistent with the recent diagnosis of HSMI in Pacific Canada<sup>8</sup> were identified during a pre-transfer government audit of an Atlantic salmon sea-pen cohort in the Johnstone Strait off the eastern coast of Vancouver Island. Of the 40 fish sampled, 11 (28%) had inflammatory heart lesions within the atrium and ventricle that were moderate (10 fish) to severe (1 fish); two fish (5%) also had moderate inflammation within the red skeletal muscle. Between July 29<sup>th</sup> and August 7<sup>th</sup>, 2016, 5 of 11 fish (45%) from the same farm were also diagnosed with moderate to severe heart inflammation characteristic of HSMI, although these samples did not have skeletal muscle inflammation. In a final sampling specifically for this study on August 19<sup>th</sup>, 2016, 3 of 20 fish (15%) had moderate to severe heart inflammation (but no moderate or severe skeletal muscle inflammation) and all 20 were qPCR positive for PRV L1 RNA with a mean load of  $9.0e^8$  ( $\pm 6.9e^8$  SEM) reverse transcribed copies per mL blood. The pathologists (HNS and GDM) who initially assessed these samples, although blinded to PRV status, reported that the lesions were similar to descriptions of HSMI as it is currently diagnosed in Norway, which was confirmed by a third pathologist (Renate Johansen; Pharmaq Analytiq, Bergen Norway) with long experience diagnosing HSMI in farmed Atlantic salmon in Norway. A summary for the histopathology scoring of heart and muscle tissues from all three pathologists was highly comparable (Supplement 1).

All fish on the affected site were progeny of North America-adapted Mowi-McConnell brood stock<sup>27</sup> and had been reared in a single freshwater facility on Vancouver Island, Canada. Fish were presumed to be PRV-free upon seawater entry because virus was not detected by qPCR within a companion cohort from the same freshwater hatchery where these fish were sourced (n=20). The first sampling and diagnosis of HSMI-like lesions was 2-3 months post seawater entry. At sea, and specific to the farm and period where the lesions were diagnosed, the site veterinarian reports that the population did not have clinical signs of disease. Total farm monthly mortality (all causes) was between 0.2 - 0.5%, which is below the industry average of ~0.83%, and feeding behavior was normal with mean growth rates in accordance with company-targeted projections.

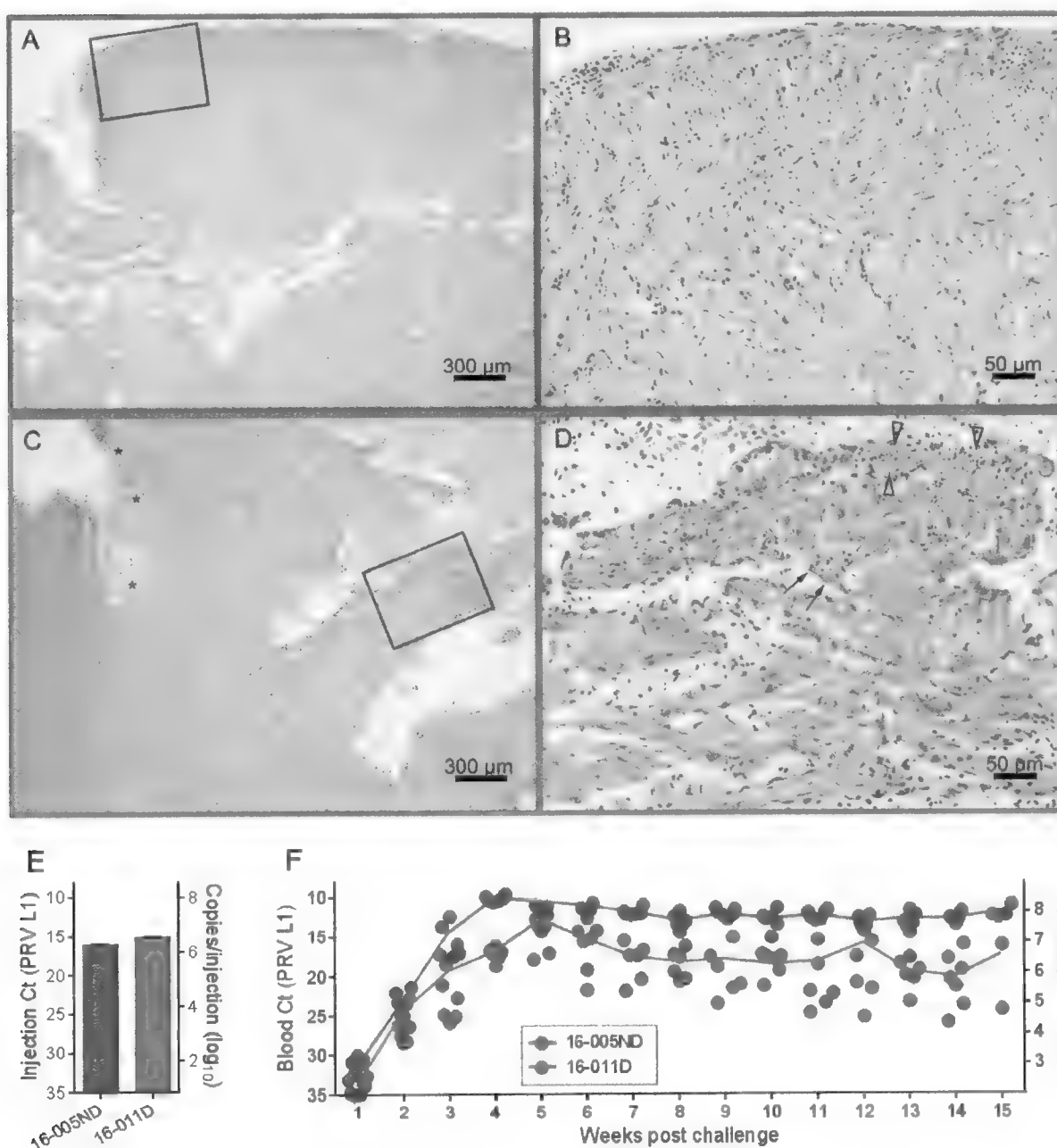
# **PRV from Atlantic salmon with and without HSMI-like lesions generate extensive and persistent infections in naïve recipients.** Naïve juvenile Atlantic salmon originating from the same brood stock and

rearing facility as the fish with HSML-like lesions described above were exposed in parallel under controlled laboratory conditions to PRV from one of two sources: (1) three fish with HSML-like heart lesions collected August 19<sup>th</sup> described above (designated 16-011D), and (2) three non-diseased fish infected by experimental challenge with PRV originating from an alternate freshwater facility with at least a three year history of PRV presence without documentation of heart or muscle inflammation; this PRV had not generated HSML in previous laboratory challenge trials<sup>13, 28</sup> (designated 16-005ND; see Methods section).

Following intra-peritoneal (i.p.) injection of similar quantities of PRV, virus replication dynamics were slightly different for the two PRV inoculates. The total quantity of PRV L1 copies per injection was  $2.0 \times 10^6$  for 16-005ND and  $3.7 \times 10^6$  for 16-011D (TaqMan PCR; Fig. 1E) and both inoculates generated substantial systemic infections. However, PRV 16-005ND rapidly disseminated and replicated to peak transcriptional loads by four weeks post challenge (wpc) with  $2.9 \times 10^8$  mean PRV L1 copies per  $\mu\text{g}$  RNA (Fig. 1F). This was estimated at  $>1.0 \times 10^{11}$  copies per mL blood based on the total RNA yield per 100  $\mu\text{L}$  blood sample. After 4 wpc, systemic PRV transcripts decreased but remained substantial, with a mean load of  $6.8 \times 10^7$  copies per  $\mu\text{g}$  RNA ( $>2.0 \times 10^{10}$  copies per mL blood) between 10 to 15 wpc. In contrast, infection with PRV 16-011D was slower to develop, with peak loads not occurring until 5 wpc and to a fivefold lesser degree (mean quantity of  $6.0 \times 10^7$  PRV L1 copies per  $\mu\text{g}$  RNA) than observed for PRV 16-005ND. Further, although the PRV 16-011D challenge generated large quantities of virus with persistent high-load systemic infections –  $3.2 \times 10^6$  mean copies per  $\mu\text{g}$  RNA, representing  $>1.3 \times 10^9$  copies per mL blood between 10-15 wpc – this persistent load was only about 5% of PRV 16-005ND.

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**Figure 1: PRV from Atlantic salmon with and without HSMI-like lesions generate extensive and persistent infections in naïve recipients.** (A, B) PRV was obtained from the blood of donor fish without heart lesions (16-005ND) or (C, D) with HSMI-like lesions (16-011D). Lesions in diseased fish included epicarditis (\*), endocardial cell hypertrophy (arrows) and small foci of myocardial necrosis (open arrowheads). Black boxes within left images outline the area shown at higher magnification in right images. (E) The quantity of PRV L1 transcripts of PRV 16-005ND and 16-011D inoculated into each naïve recipient was estimated by qPCR, and (F) the systemic blood load of PRV L1 transcripts was assessed every 7 days in recipient fish (n=6 per treatment where available). The unique relative qPCR threshold cycle (Ct) associated with each sample (dots) as well as the mean estimated quantity of L1 copies per  $\mu$ g total RNA at each time point (lines) are shown.

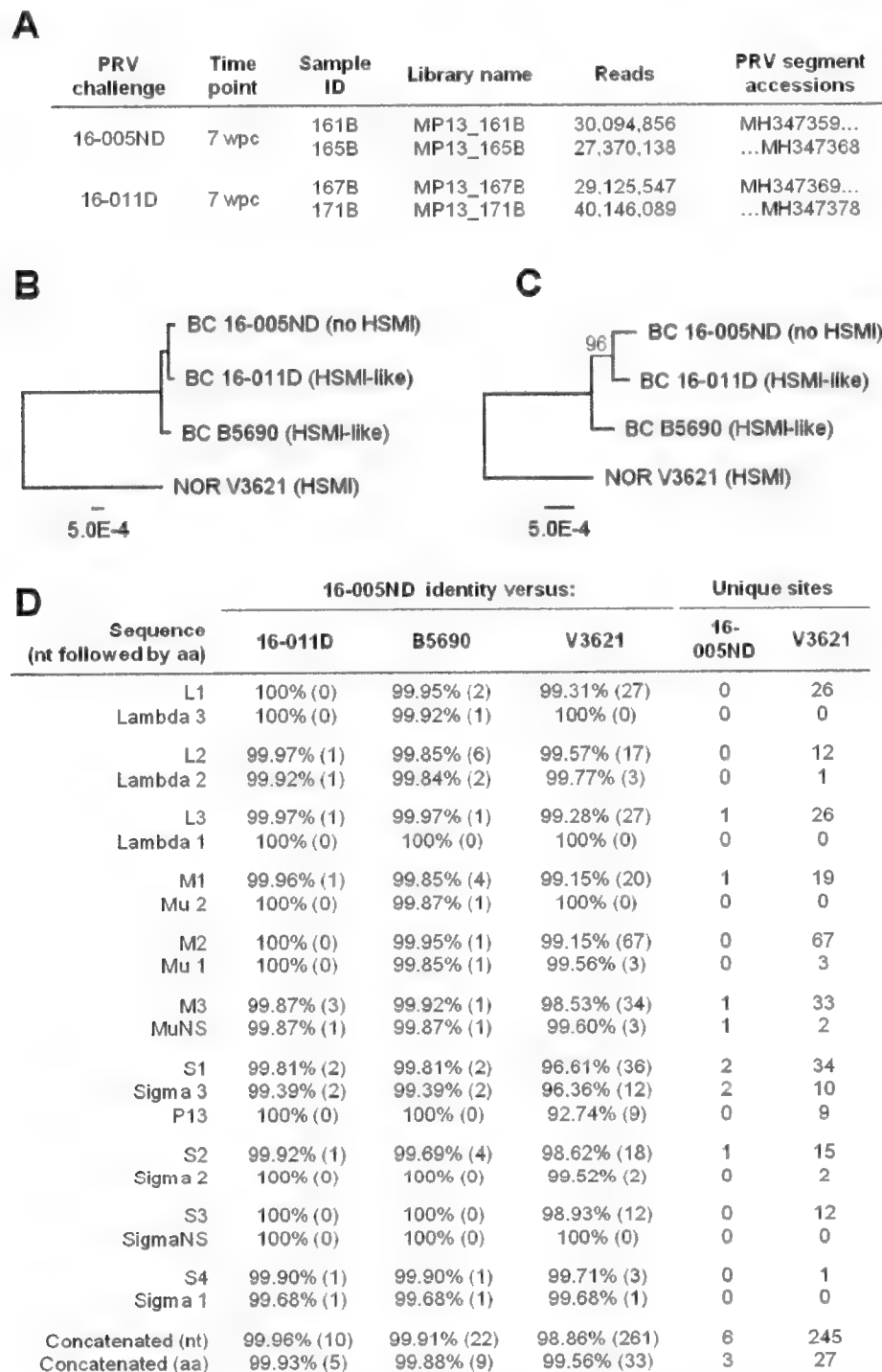
**PRV sourced from cohorts with and without HSMI-like lesions have high sequence similarity.** RNA extracted and purified from the blood of both PRV 16-005ND and 16-011D challenged fish at 7 wpc (n=2 per treatment) was subjected to next-generation RNA-sequencing (RNA-seq) to independently obtain the genomic

sequences of PRV 16-005ND and 16-011D. Following rRNA depletion and Illumina sequencing, 27-40 million reads with Phred scores >33 were generated from RNA of each fish and deposited in the NCBI Sequence Read Archive, study SRP145317. Individual SRA library accession numbers are provided in Fig. 2A. Libraries specific to either 16-005ND or 16-011D were pooled for *de novo* transcript assembly using Trinity<sup>29</sup> which identified all 10 genomic segment of PRV for both isolates. The longest Trinity assembled PRV transcript segments were deposited in Genbank; accession MH347359 – MH347378 (Fig. 2A). *De novo* assembled transcripts had 98.5% (16-005ND) and 98.9% (16-011D) concatenated coverage across all segments with respect to two previously published PRV genomes – one isolated from diseased Atlantic salmon in British Columbia during the first reported outbreak of HSMI in the region (isolate B5690)<sup>8</sup>, the other from HSMI diseased Atlantic salmon in Norway which was used to demonstrate laboratory passage of HSMI and the causal relationship of PRV in HSMI disease within Norway (NOR2012-V3621)<sup>1</sup>. This coverage incorporated 99.5% (16-005ND) and 100% (16-011D) of the protein coding sequence presented in these two published genomes where the only notable loss in coverage was the putative 128 bp (41 aa) missing 5' end of the L3 segment for 16-005ND.

Genomic sequences of 16-005ND and 16-011D have 99.96% nucleotide identity with only 10 nucleotide substitutions within the approximate 23 kb concatenated genomes. Both isolates are also highly similar (>99.9% nucleotide identity) to the B5690 isolate previously identified in BC, but are comparatively divergent (~98.9% nucleotide identity) to the Norwegian V3621 isolate (Fig. 2B-D). This pattern of divergence is similarly reflected in predicted protein translation. Specifically, V3621 has 245 unique nucleotide substitutions that results in 27 predicted amino acid changes relative to the three Canadian isolates considered in this study across the concatenated genome, with the highest proportion of translational variation being associated with the bicistronic S1 segment (Fig. 2D). However, only six nucleotide substitutions (resulting in 3 predicted amino acid substitutions) are unique to Pacific Canada PRV sourced from non-diseased Atlantic salmon (16-005ND) compared to two isolates from HSMI diseased fish in Norway and fish with HSMI-like lesions in Pacific Canada. All three of these putative amino acid substitutions (one from MuNS, two from Sigma 3) are predicted to alter protein secondary structure as identified by the EMBOSS 6.5.7 garnier plugin within Geneious 9.1.7 software. The MuNS substitution is predicted to eliminate a coil at position 386 thereby extending an alpha helix. The substitution at position 180 of Sigma 3 results in the same alteration, whereas the substitution at position 230 of Sigma 3 results in the predicted shortening of a beta-strand and lengthening the subsequent alpha helix. These structural changes are predicted with approximately 65% accuracy and the consequences (if any) for these changes in secondary structure or putative protein function are unknown.

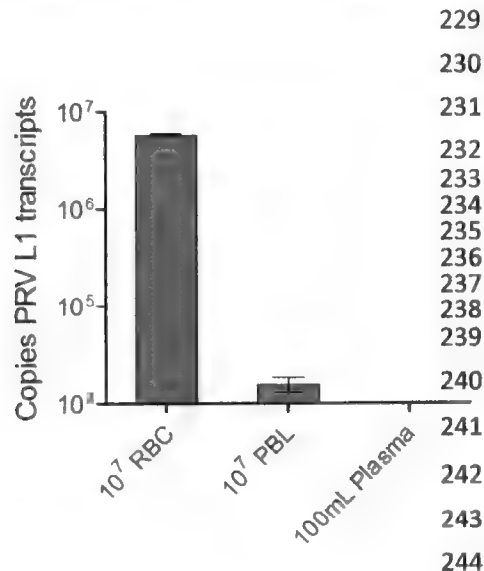
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**Figure 2: PRV sourced from cohorts with and without HSMI-like lesions have high sequence similarity.** (A) RNA-seq read libraries (n=4) were pooled specific to PRV source material (n=2) and assembled *de novo* using Trinity. (B) Concatenated PRV segments are phylogenetically compared to two previously published genomes of PRV (Pacific Canada isolate B5690<sup>8</sup> and Norway isolate V3621<sup>30</sup>). Bootstrap probabilities (percentages) are provided at branch nodes if less than 100%; scale bar indicates nucleotide substitutions per site. (C) Predicted amino acid sequences are compared in the same manner as nucleotide sequences. (D) The comparative identity between nucleotide (nt) and predicted amino acid (aa) sequences as well as the number of substitutions ( ) per alignment are provided in relation to 16-005ND. Substitutions unique to either 16-005ND (no HSMI) or V3621 (only Norwegian isolate) are also indicated.

**Systemic PRV load is associated with erythrocytes and not detectable in plasma.** At 10 wpc, a portion of blood collected from three 16-005ND PRV infected fish was separated into plasma, leukocyte, and erythrocyte fractions. By qPCR screening, virtually all PRV L1 transcripts were associated with the erythrocyte fraction during this later phase of infection (Fig. 3). Further, PRV L1 RNA was not detected in (i) plasma (limit of detection of approximately 30 copies per 100  $\mu$ L plasma screened) at the 10 wpc time point or (ii) from the plasma of any PRV-challenged fish (both 16-005ND and 16-011D) collected across all sampling time points.



**Figure 3: Systemic blood PRV load is associated with erythrocytes and not detectable in plasma.** Copy number of PRV L1 transcripts at 10 weeks post PRV 16-005ND challenge (n=3) was high within the erythrocyte red blood cell (RBC) fraction, low within the peripheral blood leukocyte (PBL) fraction, and not detectable in plasma.

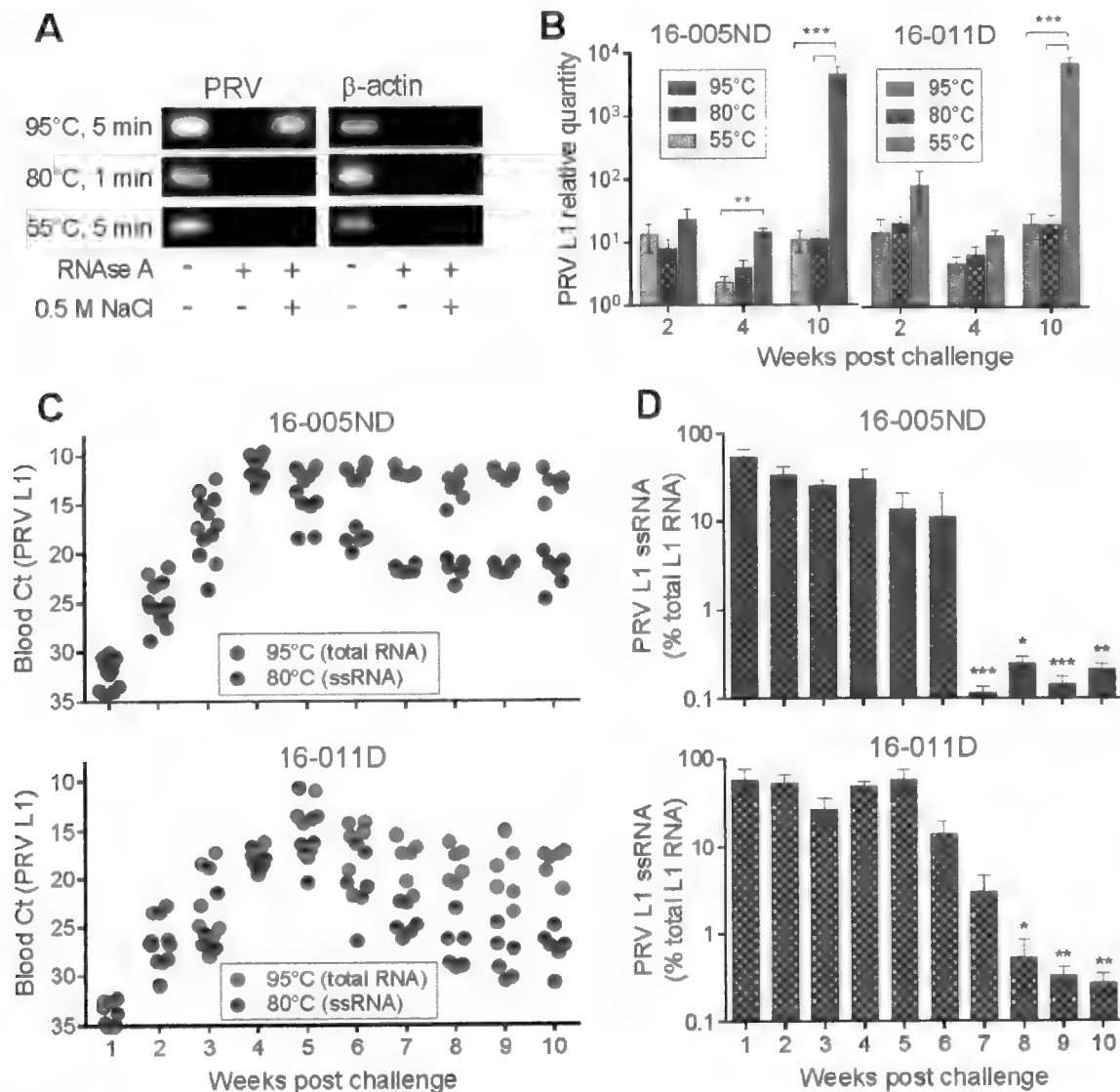
**The relative proportion of PRV genomic and messenger RNA in erythrocytes changes over the time course of infection.** Reoviruses have a dsRNA genome which is asynchronously transcribed. Positive-strand RNA is synthesized first which acts as mRNA template for viral protein translation and template for subsequent minus-strand synthesis of genomic material (gRNA)<sup>31</sup>. To better understand the timing and degree of PRV protein production versus gRNA synthesis, we developed a new method to differentially quantify PRV single-stranded (mRNA) and double-stranded (gRNA) RNA using qPCR. Because recombinant reverse transcriptase such as MultiScribe™ selectively target ssRNAs during cDNA synthesis, we hypothesized that PRV dsRNA segments would not be detected by qPCR unless they were denatured into single-strands prior to reverse transcription and that this would require temperatures above 90°C based on sequence-specific melting point estimations (<http://www.endmemo.com/bio/tm.php>). By this reasoning, exposure to low temperatures (i.e. below 90°C) for the removal of secondary structure prior to reverse transcription would result in only single-stranded PRV (mRNA) detection by qPCR. Experimental testing confirmed this hypothesis to be correct, as the dsRNA genomic component was only measureable in those samples denatured at 95°C and not following exposure to 55 or 80°C (Fig. 4A). Capitalizing on the ability to differentiate mRNA vs gRNA via differential temperature regimes, we analyzed early (2 wpc), peak (4 wpc), and late (10 wpc) stages of PRV infection. Utilizing two temperatures (55° and 80°C) below the threshold needed to denature dsRNA, the relative proportional quantity of PRV L1 ssRNA transcripts was not significantly different at either temperature across all three time points. Yet, when measuring both mRNA and gRNA in the denatured samples (95°C), the fraction of the gRNA differed significantly dependent upon sample time point. At 2 and 4 wpc the ssRNA component constituted approximately 50 ( $\pm$  40)% of the total RNA while at 10 wpc the ssRNA component was significantly reduced to represent 0.1 to 0.7% of the total PRV



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265 RNA load with gRNA accounting for the vast majority (Fig. 4B). Subsequent targeting of total and ssRNA PRV L1  
266 transcripts in all blood samples collected between 1 and 10 wpc confirmed that during early and peak replication,  
267 single-stranded mRNA typically represented 10-90% of the total PRV transcriptional load; but, after approximately  
268 5-6 wpc, the quantity of single-stranded mRNA quickly became proportionally less. By 7-8 wpc, PRV ssRNA  
269 represented only 0.1-1.0% of the total systemic transcriptional load which was significantly less than during early  
270 (1wpc) infection (Fig. 4C and D). This pattern of expression was strikingly similar following challenge with either  
271 PRV 16-005ND or 16-011D.



**Figure 4: The relative proportion of PRV genomic and messenger RNA in blood changes over the time course of infection.** (A) RNA from PRV 16-005ND infected fish at 10 wpc was used to validate differential detection of PRV L1 ssRNA (mRNA) and dsRNA (gRNA) transcripts by qPCR. Total RNA exposed to either no enzyme, RNase A, or RNase A in 0.5M sodium chloride (which selectively protects dsRNA but not ssRNA from RNase A degradation<sup>32</sup>) was heated at 55°C for 5 min, 80°C for 1 min, or 95°C for 5 min prior to reverse transcription and 30-cycles of qPCR. PRV dsRNA template was only present following 95°C denaturation as seen in cropped gel images (for Ct values see Supplement 1). (B) The relative quantities (scaled to the minimum value per time point) of PRV L1 at 2, 4, and 10 wpc for both PRV 16-005ND and 16-011D are compared between pre-amplification denaturation temperatures. The proportion of dsRNA to ssRNA significantly increased at 10 wpc in both PRV challenged groups (\* p < 0.05; \*\* p < 0.01, \*\*\* p < 0.001). (C) The shift toward higher proportions of PRV dsRNA in



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282 blood began around 5-6 wpc; (D) where the amount of ssRNA became significantly reduced after 7-8 wpc relative to 1wpc  
283 proportional quantities.  
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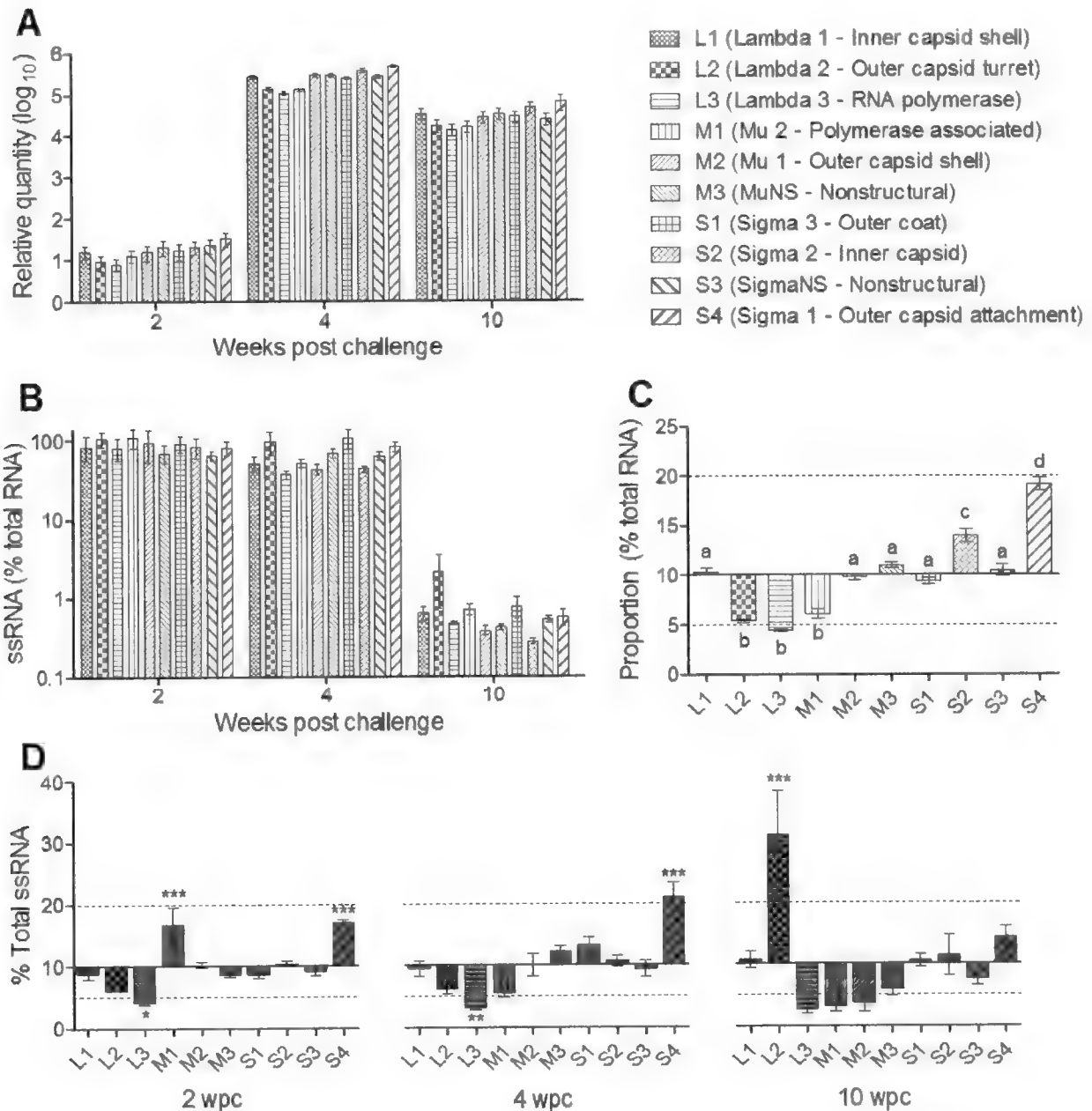
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286 **Expression of PRV segments is temporally similar but with slight proportional variation.** To  
287 compare and contrast the relative load for each of the 10 PRV genomic segments following experimental infection,  
288 SYBR™ green qPCR assays were designed against each of the 10 genomic segments of PRV and used to assess  
289 relative quantities of each segment in the blood of PRV 16-005ND challenged fish at early (2 wpc), peak (4 wpc)  
290 and persistent (10 wpc) phases of infection. This identified that relative quantities of all 10 segments of PRV 16-  
291 005ND were not significantly different at any of the three time points analyzed even though the total PRV RNA load  
292 encompassed an approximate  $10e^4$  fold change in systemic load over this time (Fig. 5A); indicating the proportion  
293 of total PRV RNA represented by each segment was conserved during all three infection stages. However, the  
294 proportion of each segment contributing to the total PRV RNA independent of time was not equal; this variation was  
295 less than twofold but statistically significant ( $p < 0.05$ ). Specifically, L2, L3 and M1 were proportionally  
296 underrepresented whereas S1 and particularly S4 were overrepresented relative to the other segments (Fig. 5C).

297 Selective ssRNA targeting techniques previously validated for L1 were also applied to each of the other 9  
298 segments in this dataset. In a similar pattern to L1 transcription, the ssRNA component of all 10 PRV segments was  
299 relatively high (~10-90%) during early and peak infection but became reduced (nearly all <1%) following the  
300 transition to late-stage persistent infections (Fig. 5B). Nevertheless, significant proportional variation in ssRNA  
301 quantity occurred for some segments over the course of infection. In comparison to segment L1 ssRNA, which  
302 maintained stable expression across all three time points (mean  $9.7\% \pm 0.7$  SEM of total ssRNA), segment L3  
303 ssRNA was proportionally decreased whereas segment S4 was proportionally increased during early and peak  
304 infection. Segment M1 ssRNA was also proportionally increased during early infection, whereas segment L2 ssRNA  
305 only became proportionally increased during the late persistent phase (Fig. 5D). L1, M2, M3, S1, S2 and S3  
306 maintained relatively stable proportional ssRNA expression across all three time points; and even though L2, L3,  
307 M1 and S4 had significant proportional variation compared to the other stable segments, their variation was mostly  
308 encompassed within a twofold change relative to complete segment equality (10% each).

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**Figure 5: Expression of PRV segments in host blood is temporally similar but with slight proportional variation.** (A) The relative quantity (scaled to the minimum value) of each PRV RNA segment in the blood of 16-005ND challenged fish was statistically similar at 2, 4 or 10 wpc. (B) The single-stranded mRNA contribution to total PRV load was also similar between segments at each time point. However, (C) the cumulative proportional contribution of L2, L3, and M1 was significantly less, whereas S2 and S4 was significantly more, relative to all other segments independent of time (letters indicate significant groupings at  $p < 0.05$ ). (D) Total proportional contributions of total single-stranded mRNA expression also varied between segments, but was not consistent over time points (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). Dotted lines at 5 and 20% provide reference of a twofold deviation away from complete proportional equality (10%).

**Persistent late-stage PRV infections remain highly infectious by i.p. injection but have reduced ability for infecting naïve cohabitants.** The limited quantities of PRV single stranded mRNA at 10 wpc

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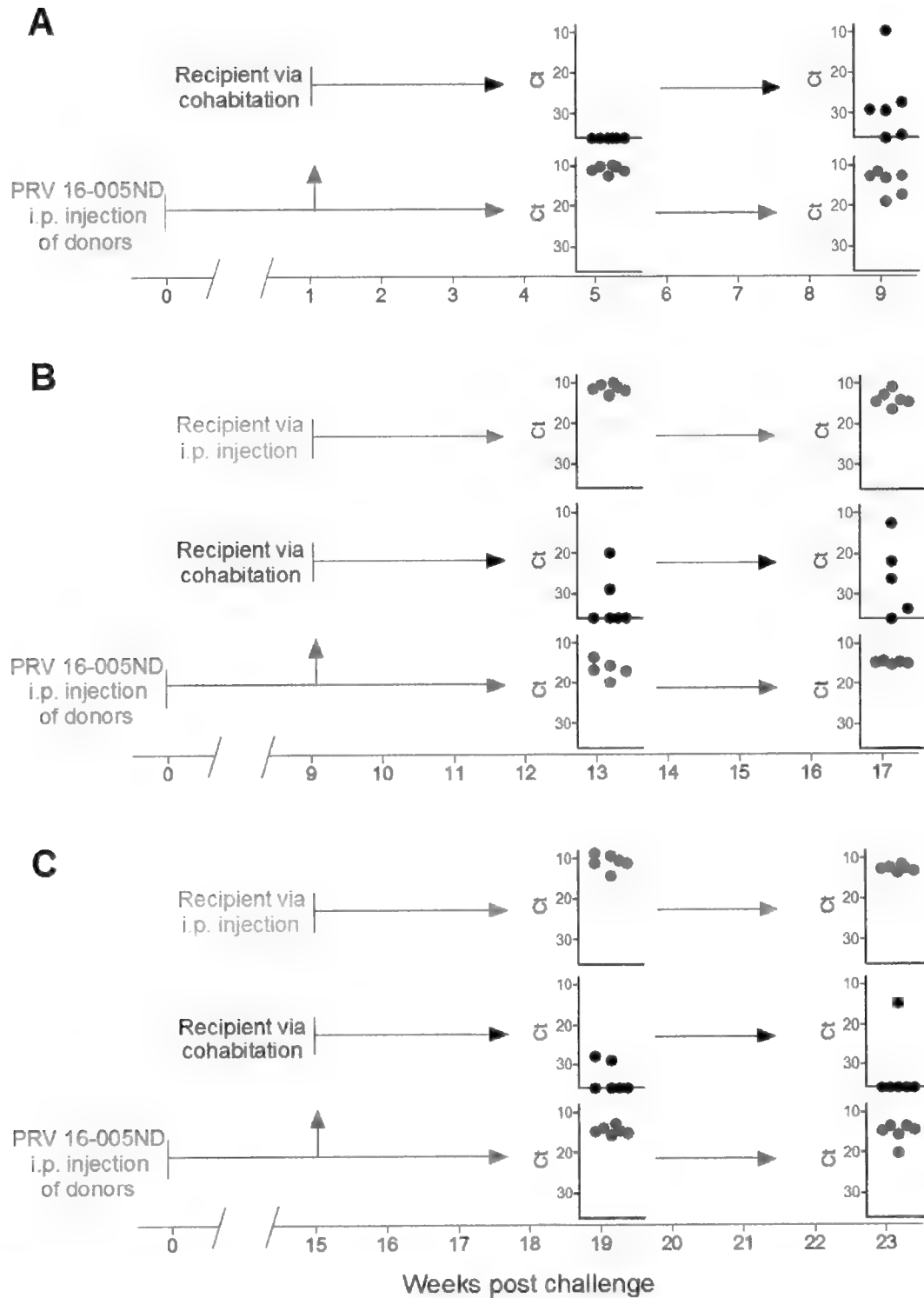
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indicated that viral replication had become substantially reduced. To determine the transmission potential of PRV during different stages of infection, we initiated a third challenge trial where three groups of naïve fish ( $n=15$  per group) were injected with PRV 16-005ND from which viral passage was attempted by either cohabitation (1:1 shedder to naïve fish ratio) or by i.p. injection (100  $\mu$ L blood homogenate diluted 1:10 in saline) to naïve recipients introduced at either 1, 9, or 15 weeks post challenge (Fig. 6). Fish injected with PRV 16-005ND had PRV infection dynamics similar to those observed in the first challenge. Viral loads were high at 5 wpc ( $6.2 \times 10^7$  mean copies per  $\mu$ g RNA), were slightly less at 9 wpc, and stabilized at reduced but still substantial loads at 13, 17, 19 and 23 wpc ( $4.9 \times 10^6$  mean copies per  $\mu$ g RNA). Passage of virus to cohabitants introduced soon after the primary injection exposure (1wpc) provides evidence that natural shedding might be minimal in this early period of infection as PRV L1 RNA could not be detected in sentinel fish after 4 weeks of cohabitation. Even after 8 weeks of cohabitation, when 5 of the 6 sampled fish were positive for PRV L1 RNA, the systemic loads were still relatively low ( $<1 \times 10^3$  copies per  $\mu$ g RNA) in all but one fish (Fig. 6A); suggesting an early stage of dissemination.

PRV passage via cohabitation was successful during late stage infections. At least a portion of naïve cohabitants introduced at either 9 or 15 wpc became infected during 8 weeks of cohabitation (Fig. 6B and C). However, these infections tended to be slow to develop and more fish became infected when introduced at 9 wpc (7/12) than when introduced at 15 wpc (3/12). This might be a result of less infectious virus being shed in chronically infected fish than from fish at or near peak infection. Nevertheless, passage of virus during this late persistent phase was readily accomplished via i.p. injection of blood homogenate into naïve recipients which generated high-load systemic infections in 100% of fish within 4 wpc. Indeed, injection of this late-stage material into naïve recipients yielded infection dynamics nearly identical to the first challenge with PRV 16-005ND, which had been harvested for passage at 4 wpc just when peak loads had been reached (Fig. 6B and C).

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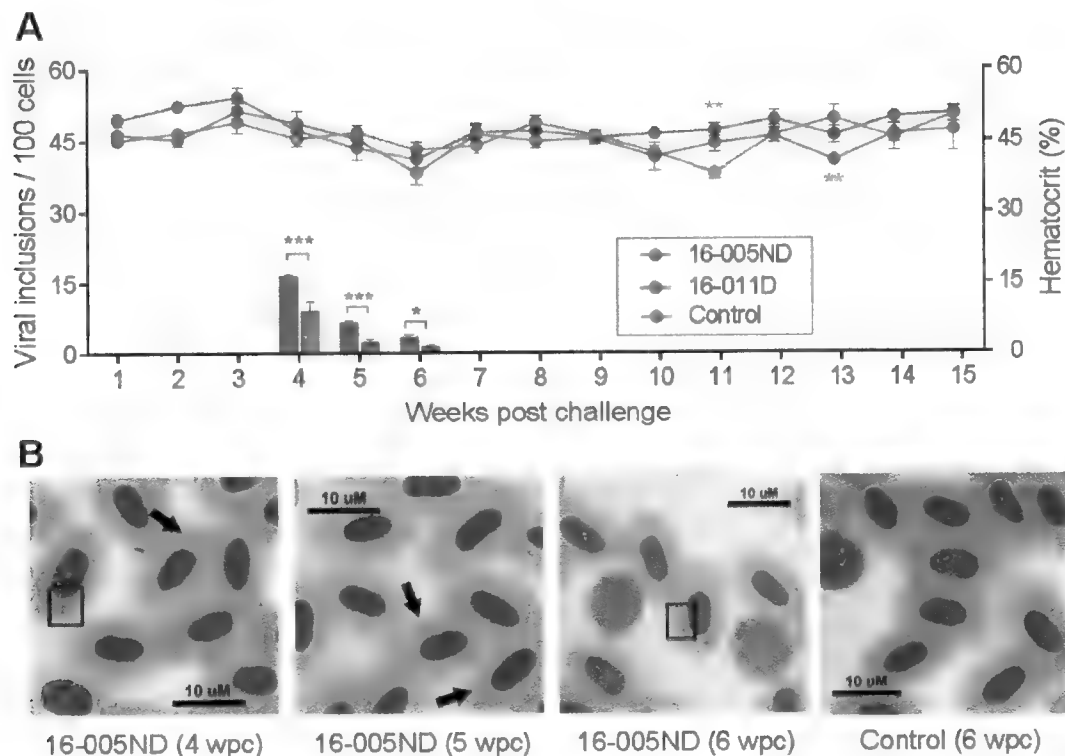


**Figure 6: Persistent late-stage PRV blood infections remain highly infectious by i.p. injection but have reduced ability for infecting naïve cohabitants.** Passage of PRV 16-005ND from i.p. injected donor fish into naïve recipients by either i.p. injection or cohabitation was attempted at (A) 1, (B) 9, and (C) 15 wpc. After a period of 4 or 8 weeks following each attempted passage, blood of both recipients and donor fish (n=6 per group) was screened for total PRV L1 transcripts by qPCR. The unique relative qPCR threshold cycle (Ct) associated with each sample is presented. Samples plotted on x-axes indicate a lack of detection for PRV L1 transcripts in each instance (no Ct).

# **PRV sourced from cohorts with and without HSMI-like lesions cause transient erythrocytic inclusions but not anemia in naïve recipients.**

Despite PRV reaching extreme ( $>10^9$  PRV L1 copies per mL) systemic blood loads following PRV 16-005ND and 16-011D injection challenges that were almost exclusively associated with erythrocytes, neither PRV 16-005ND nor 16-011D caused a notable reduction in hematocrit (Fig. 7A). Relative to time matched controls, significant differences in hematocrit were uncommon (two sample sets) and inconsistent. Hematocrit was greater at 11 wpc in PRV 16-005ND challenged group and less at 13 wpc in PRV 16-011D challenged group and, in both instances, did not deviate beyond the range of control fish (38-50%) where all values were well above an approximate 25% hematocrit threshold previously estimated to represent functional anemia in salmon<sup>33</sup>.

Transient viral inclusion bodies were observed by pinacyanol chloride staining between four and six weeks post challenge in blood smears of both PRV 16-005ND and 16-011D infected fish. Up to 15% of erythrocytes had inclusions and fish challenged with PRV 16-005ND had slightly higher quantities compared to those infected with 16-011D (Fig. 7A). Single, large spherical viral inclusions occurred at 4, 5, and 6 wpc; however, at 4 wpc when inclusions were first identified, affected erythrocytes from both challenge groups also had high prevalence of clusters of smaller inclusions. These primarily occurred in darkly stained (presumably immature) erythrocytes (Fig. 7B). At 5 wpc, large spherical inclusions predominated. By 6 wpc, inclusions appeared to be breaking up, as clusters of smaller inclusions again became dominant. No viral inclusions were observed at any other time point during these challenge trials.



**Figure 7: PRV sourced from blood of cohorts with and without HSMI-like lesions cause transient erythrocytic inclusions but not anemia in naïve recipients.. (A)** The trend in mean ( $\pm$ SEM) hematocrit (lines) for both PRV 16-005ND and 16-011D challenged fish in comparison to time-matched experimental controls did not suggest anemia in any treatment group. Erythrocytes with viral inclusions (bars) were observed between 4 and 6 wpc and were more prevalent in 16-005ND challenged fish (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). T. (B) Erythrocyte cytoplasmic inclusions were defined as either a single, large spherical body (arrows) or as a cluster of smaller globular bodies (boxed) with moderate to dark staining.

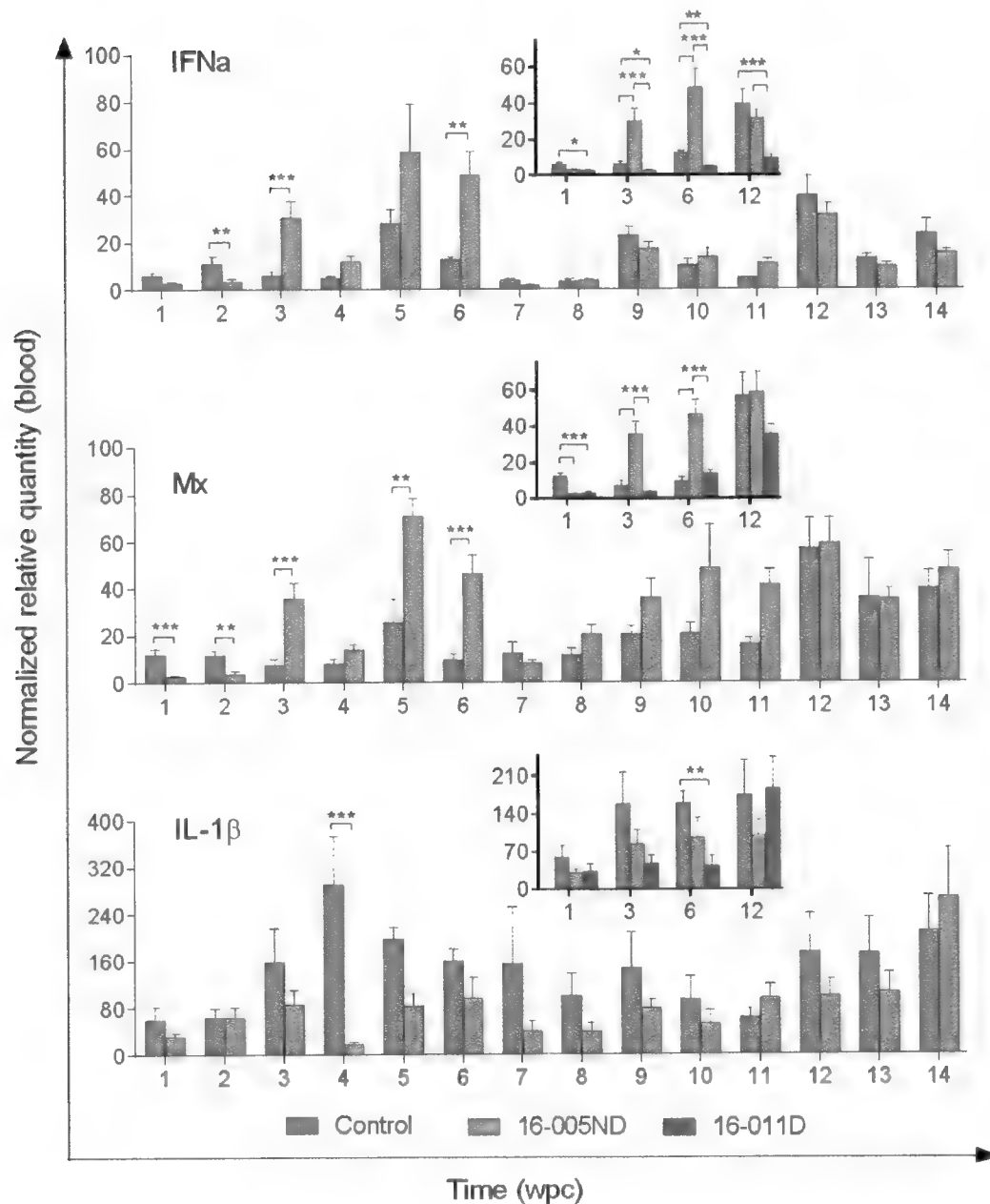
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379 **Systemic host transcriptional responses against PRV are minor, transient, and possibly load**  
380 **dependent.** To identify if and when PRV is recognized by host blood cells, transcription of two classic viral  
381 recognition/antiviral defense genes were monitored during the PRV challenge trials: (i) *IFN $\alpha$* , a type-I interferon of  
382 salmon which is generated in response to viral recognition in nearly all cell types<sup>34</sup>, and (ii) *Mx*, an antiviral response  
383 element triggered by cellular recognition of interferon<sup>35</sup>. Both genes were transcriptionally down-regulated during  
384 early infection (1-2 wpc) in both PRV 16-005ND and 16-011D infected fish relative to time-matched controls;  
385 however, as PRV infections progressed, significant induction of both *IFN $\alpha$*  and *Mx* occurred in 16-005ND but not  
386 16-011D challenged fish (approximately 4-5 fold) when virus reached at or near peak loads (3 and 6 wpc) (Fig. 8).  
387 Neither *IFN $\alpha$*  nor *Mx* transcription was significantly up-regulated following 16-011D challenge at any of the time  
388 points analyzed. The lower systemic viral loads generated during peak 16-011D infection provides evidence that  
389 the response observed in 16-005ND was load dependent. No significant change in *IFN $\alpha$*  or *Mx* transcriptional  
390 expression occurred during the late stages of PRV infection (7-15 wpc) in either 16-005ND or 16-011D challenges  
391 relative to time-matched controls. Further, monitoring of *IL-1 $\beta$*  transcription, an important cytokine involved in  
392 inflammatory process of vertebrate and non-vertebrate animals including salmon<sup>36</sup>, also did not demonstrate  
393 significant systemic induction in blood of either PRV 16-005ND or 16-011D infected fish at any time point following  
394 challenge (Fig. 8). Rather, if any change occurred, it appeared that *IL-1 $\beta$*  transcription was slightly reduced in some  
395 instances relative to controls.

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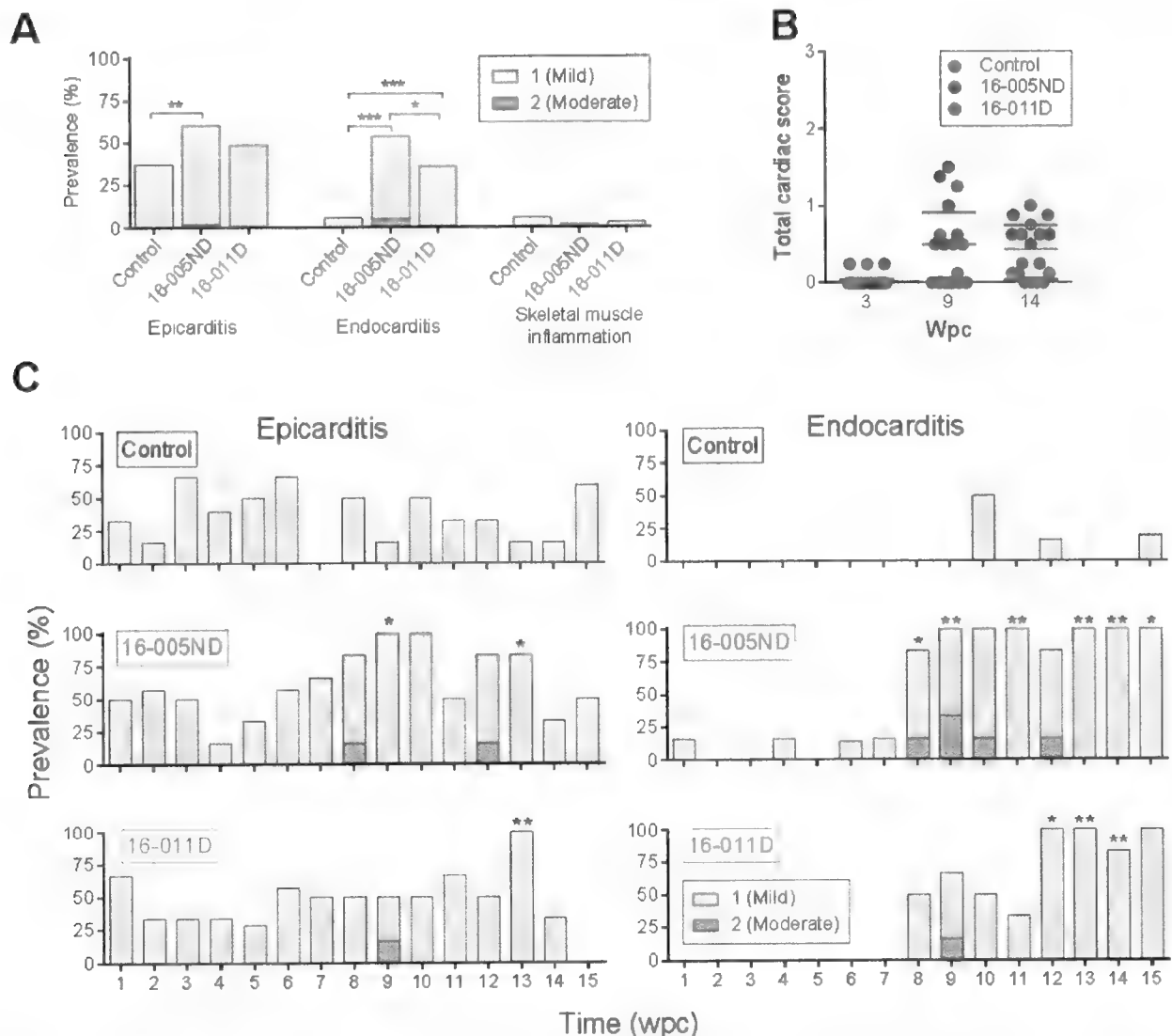
**Figure 8: Systemic host transcriptional responses in blood against PRV are minor, transient, and possibly load dependent.** The gene expression of *IFNa*, *Mx*, and *IL-1β* (proteins involved in viral response, antiviral, and inflammatory pathways, respectively) were monitored in 16-005ND challenged and control fish at weekly intervals and in 16-011D challenged fish at 1, 3, 6, and 12 wpc (see insert graphs). Significant (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ) increased expression from time-matched controls of *IFNa* and *Mx* but not *IL-1β* occurred when PRV 16-005ND load was near its peak (3-6 wpc). In each instance, expression was normalized to the stable expression of  $\beta$ -actin and scaled to the minimum observed value.

**Minor heart inflammation occurs during persistent late-stage PRV infections independent of donor fish disease status.** All heart and red and white skeletal muscle samples collected during this study were examined by pathologists GDM and/or HNS with a subset collected at 3, 9 and 14 wpc also evaluated by a reviewing pathologist (Renate Johansen, Pharmaq Analytiq). In all instances, pathologists were blinded to PRV

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exposure status and lesions were scored according to severity (0 – none, 1 – mild/small amount, 2 – moderate, or 3 – severe/abundant) similar to methods applied in previous HSMI studies<sup>1, 8, 22, 37</sup> (Supplement 1). Mild lymphohistiocytic epicarditis was common in hearts of control fish during this study with a mean prevalence of 37% ( $\pm 5\%$  SEM) over the 15 week trial. Nevertheless, the prevalence of epicardial inflammation was greater in PRV 16-005ND challenged fish (mean  $60 \pm 5\%$  SEM) which was accompanied by a significant increase in the overall median endocarditis severity score relative to controls (Fig. 9A). The prevalence of epicarditis in 16-011D challenged fish was also nominally greater than in controls (mean  $48 \pm 6\%$  SEM), but the change in median severity score was not significant.



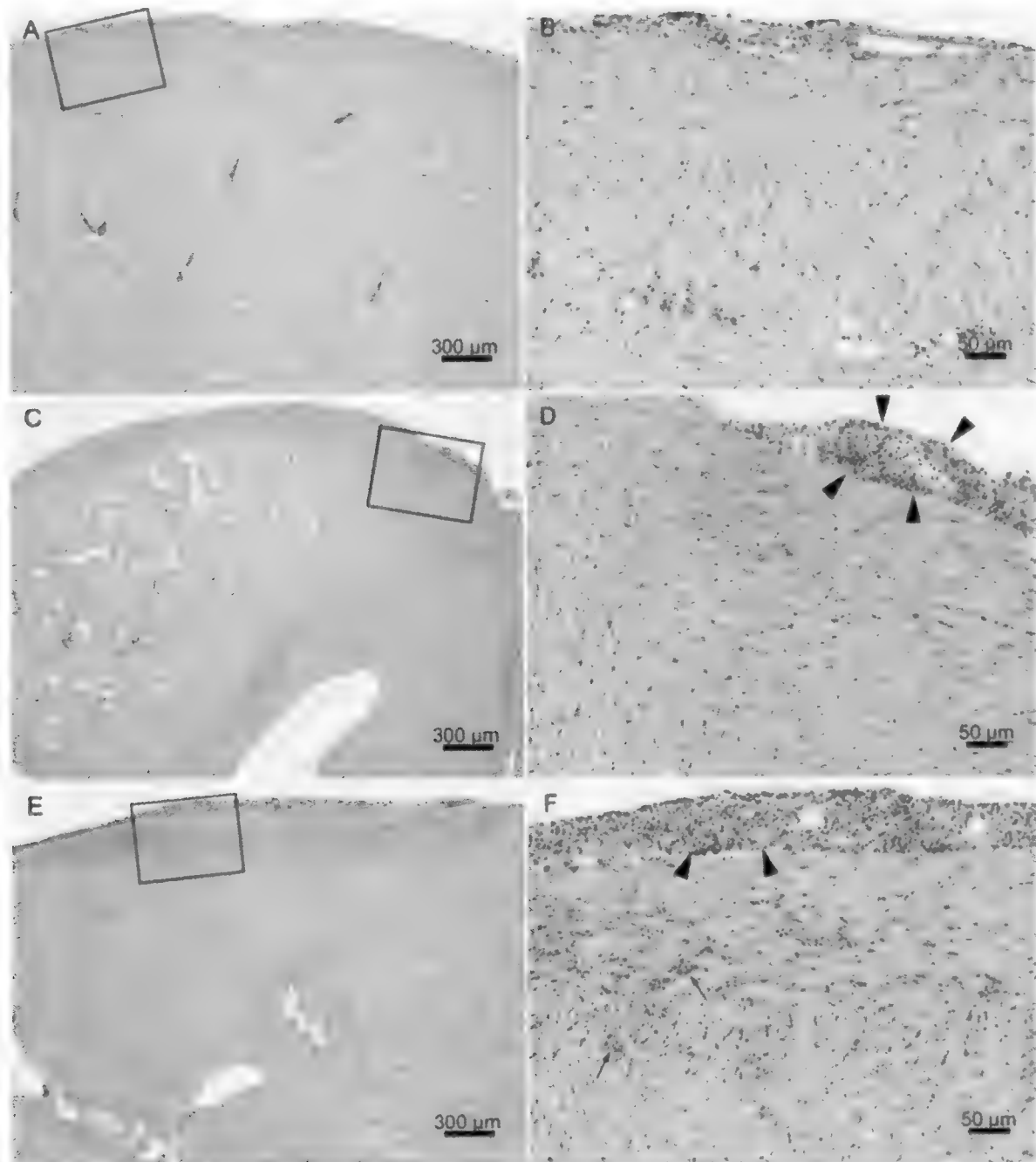
**Figure 9: Minor heart inflammation occurs during persistent late-stage PRV infections independent of donor fish disease status.** (A) The cumulative prevalence of epicarditis, endocarditis and skeletal muscle lesions identify significantly increased prevalence of heart but not muscle lesions in PRV challenged fish within 15 wpc (\*  $p < 0.05$ ; \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ). However, (B) the overall heart severity scores (combined mean inflammatory score for the atrium, epicardium, compactum and spongiosa) did not progress beyond a mean value of 1 (mild) in any treatment group at 3, 9, or 14 wpc. (C) Prevalence and severity of heart lesions assessed at 7 day increments throughout the trial ( $n=6$  per time point) also identified mild epicarditis and endocarditis which at some time points were significantly elevated relative to controls. The occasional occurrence of lesions of moderate severity was only observed in PRV infected groups.



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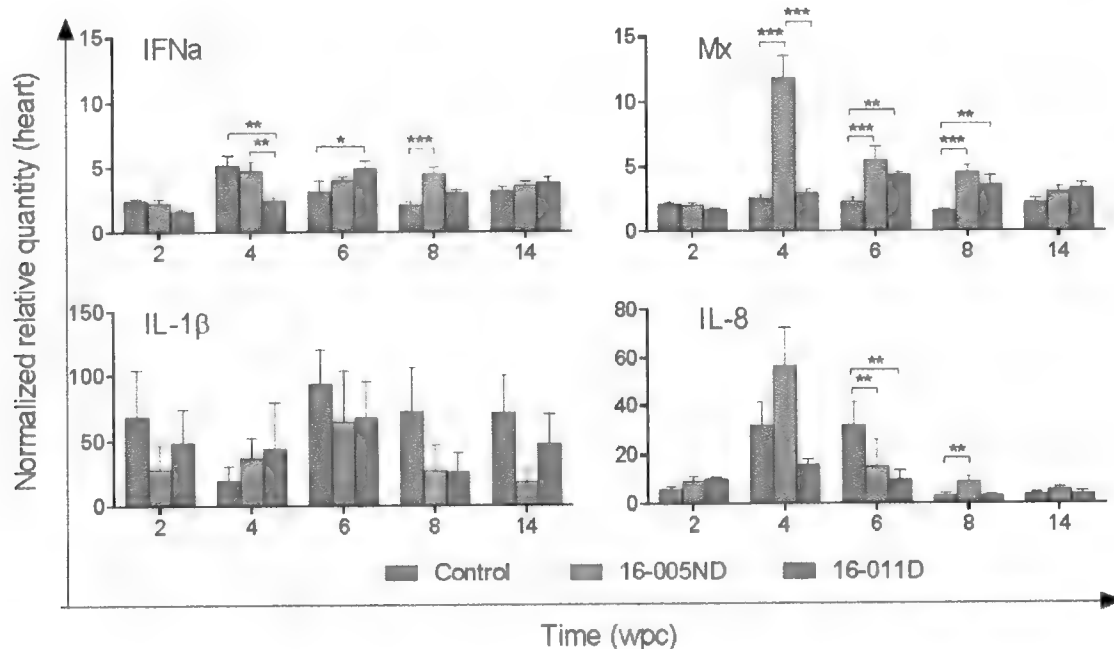
Endocardial tissues provided a clearer association between PRV and heart inflammation, where both 16-005ND and 16-011D challenged fish had significantly greater median severity scores for lymphohistiocytic endocarditis compared to controls (Fig. 9A). This mainly occurred between 8 and 15 wpc in both challenge trials representing the post-peak persistent phase of PRV infection within these populations (Fig. 9C). PRV was not associated with skeletal muscle inflammation, and of the 10 (out of 270) fish with mild skeletal muscle inflammation (all cases were mild), 5 were from control, 2 were from 16-005ND and 3 were from 16-011D challenged populations.



**Figure 10: Complete range of histopathology in PRV 16-005ND and 16-011D infected fish encompassed no, low and moderate heart inflammation.** Histopathology of hearts from PRV-injected and control fish 8 week post-challenge identified

(A, B) hearts with no microscopic lesions such as in control fish #184, (C, D) hearts with mild, focal, lymphohistiocytic epicarditis (arrowheads) such as in 16-011D challenged fish #191, or (E, F) heart with either moderate lymphohistiocytic epicarditis (arrowheads), and/or endocarditis (arrows) such as from 16-005ND challenged fish #187. Note that fish #187 was the only fish in this study which had both moderate lymphohistiocytic epicarditis and endocarditis. Black boxes in the left column images outline the area shown at higher magnification in right column images; hematoxylin and eosin stain.

Interestingly, fish challenged with PRV 16-005ND (which came from a non-diseased population) developed greater prevalence of heart inflammation than fish challenged with PRV 16-011D (sourced from fish with subclinical HSMI) (Fig. 9A). However, the overall severity of heart inflammation was generally mild in both PRV challenges (Fig. 9B), with typical heart tissues having no evidence of inflammatory lesions or only minor foci of inflammation (Fig. 10A-D). Indeed, the overall severity of heart lesions in PRV challenged fish (combined mean inflammatory score for the atrium, epicardium, compactum and spongiosa) was minor even during the period of approximate highest prevalence and severity (9 wpc), which is below the severity threshold previously used to categorize an HSMI disease state (i.e., minimum total heart severity score  $>1.5 - 2$ )<sup>8,11</sup>. One fish sampled during this study at 8 wpc (a PRV 16-005ND challenged fish) had both moderate epicarditis and endocarditis (Fig. 10E and F). However, even in this most extreme instance, the severity had not yet progressed into notable myocardial necrosis. The relatively minor impact of inflammation within heart tissues during this trial was further supported by a general lack of transcriptional induction for the inflammatory cytokine *IL-1 $\beta$*  or inflammatory chemokine *IL-8* within PRV infected hearts (Fig. 11). Also, mild but significant antiviral responsiveness in heart tissues as demonstrated by *Mx* transcription was observed in response to both 16-005ND and 16-011D PRV challenge during the late-peak/early-persistent phase of infection, which only partially overlapped the timing or severity of heart inflammation.



**Figure 11: Minor and transient antiviral but not inflammatory transcriptional is induced in heart tissues following PRV infection.** The transcriptional expression of *IFNα* and *Mx*, two classic viral response elements, had minor and transient up-regulation in response PRV. Neither the inflammatory cytokine *IL-1 $\beta$*  nor the inflammatory chemokine *IL-8* had biologically relevant up-regulation within PRV infected heart tissues at the time points analyzed. Gene expression was normalized to the stable expression of  $\beta$ -actin and scaled to the minimum observed value.

## Discussion

First identified in 2010, PRV represents the newest member assigned to the *Orthoreovirus* genus<sup>18</sup>. Although *Orthoreoviruses* are almost certainly the most well-studied within the *Reoviridae* family, there is considerable diversity in pathogenicity and disease association within the genus for which much is still unknown<sup>38</sup>. PRV adds yet further complexity by associating functional and genetic commonalities to both the Aquareoviruses and Orthoreoviruses while remaining phylogenetic and functionally distinct<sup>18, 39</sup>.

In Norway, most commercial Atlantic salmon become PRV positive, but only some develop HSMI. This does not appear to be dependent on systemic PRV load, and it is not clear why some farms experience high losses due to HSMI while others do not. Nevertheless, clinical outbreaks of HSMI in farmed Atlantic salmon of Norway are reasonably common<sup>10, 11, 18, 40</sup>, and laboratory challenge trials have demonstrated a clear ability for PRV to cause severe heart lesions<sup>1</sup>. Indeed, laboratory challenges trials in Norway routinely generate severe heart lesions in accompaniment with occasional skeletal muscle lesions that are similar to lesions observed in diseased salmon farms<sup>11, 19, 20, 21, 41</sup>.

The results of our study expand our understanding of a strikingly divergent relationship regarding PRV and its association with disease in Pacific Canada. PRV also appears to be highly prevalent in farmed Atlantic salmon of Pacific Canada<sup>5</sup>; yet, only rare subclinical cases of farm-level HSMI-like pathology have been reported and a clinical outbreak of HSMI as described in Norway<sup>10, 11</sup> has never been described. Here we diagnosed one case of HSMI-like disease from a pre-transfer government farm site audit, with up to five additional cases being identified between 2011 and 2013<sup>8</sup>. Even if all isolated cases of Idiopathic cardiopathy observed during these audits were presumed to be HSMI, it would constitute approximate 2% prevalence within dead and dying farmed Atlantic salmon of British Columbia. Because annual mortality among farmed Atlantic salmon in Pacific Canada is about 10-15%, HSMI-like lesions would thus (at most) be associated with only about 0.3% annual mortality across the industry. In a laboratory setting, PRV from Pacific Canada has failed to cause severe heart lesions or any severity of skeletal muscle inflammation despite establishing high-load blood infections<sup>13</sup>. Here we confirm these findings using PRV from two different commercial sources in Pacific Canada which similarly replicated to high loads following experimental infection.

The consistent dissimilarity in disease outcome following PRV infection in Atlantic salmon of Norway versus Pacific Canada leads to the rather straightforward hypothesis that something within the host-pathogen-environment dynamic is different between these two geographic regions. One potential difference is that genetic divergence in PRV between these two regions is sufficient to result in altered virulence. The sequencing of PRV genomic material from two discrete sources in this study supports previous observations that PRV appears to have low phylogenetic diversity within farmed Atlantic salmon of Pacific Canada yet is relatively distinct from Norwegian isolates<sup>14, 42</sup> (Fig. 2B).

Small genomic changes can be enough to drastically alter the virulence of a virus, such as in the HPR0 variant within infectious salmon anemia virus (ISAV)<sup>43</sup> or VP2 variants of infectious pancreatic necrosis virus (IPNV)<sup>44</sup>, and it is possible that one or more of the three putative amino acid changes unique to the PRV isolated from non-diseased fish used in this study could be held responsible for an increase in virulence. However, as pointed out previously<sup>13</sup>, altered virulence associated with genetic viral variation are almost always accompanied by

distinct tropisms and/or altered replication kinetics. For example, HPR0 variants of ISAV have altered tissue tropisms (gill specific rather than systemic)<sup>45</sup> and low-virulent IPNV variants are correlated with reduced *in vivo* loads compared to high virulence strains<sup>46</sup>. Similar differential tropisms or replication kinetics do not appear to be evident when comparing PRV sourced from cohorts with (16-011D) and without (16-005ND) HSMI-like lesions in our study. Further, HSMI-like disease could not be passed from field-infected fish into a virtually identical cohort of Atlantic salmon held in a laboratory setting. We therefore hypothesize that at least in Pacific Canada, the ability for PRV to cause HSMI or any form of severe heart lesions cannot be attributed to a genetic variance of the virus alone.

It is nevertheless possible that the reported instances of HSMI-like disease in Pacific Canada, although sharing similar microscopic lesions, were not generated by the same mechanism(s) as in Norway. For example, perhaps PRV was contributing to and exacerbating HSMI-like disease in farmed Atlantic salmon of Pacific Canada that was initiated by alternate or synergistic factors. By this reasoning, any of the 27 putative amino acid changes observed in this study between the Canadian and Norwegian strains of PRV could act alone or in concert to produce an altered state of virulence responsible for the rather striking prevalence discrepancy for HSMI in these two countries. It therefore becomes important to consider the phenotypic characteristics of PRV following analogous laboratory challenge trials conducted in Norway and Canada which may support this hypothesis.

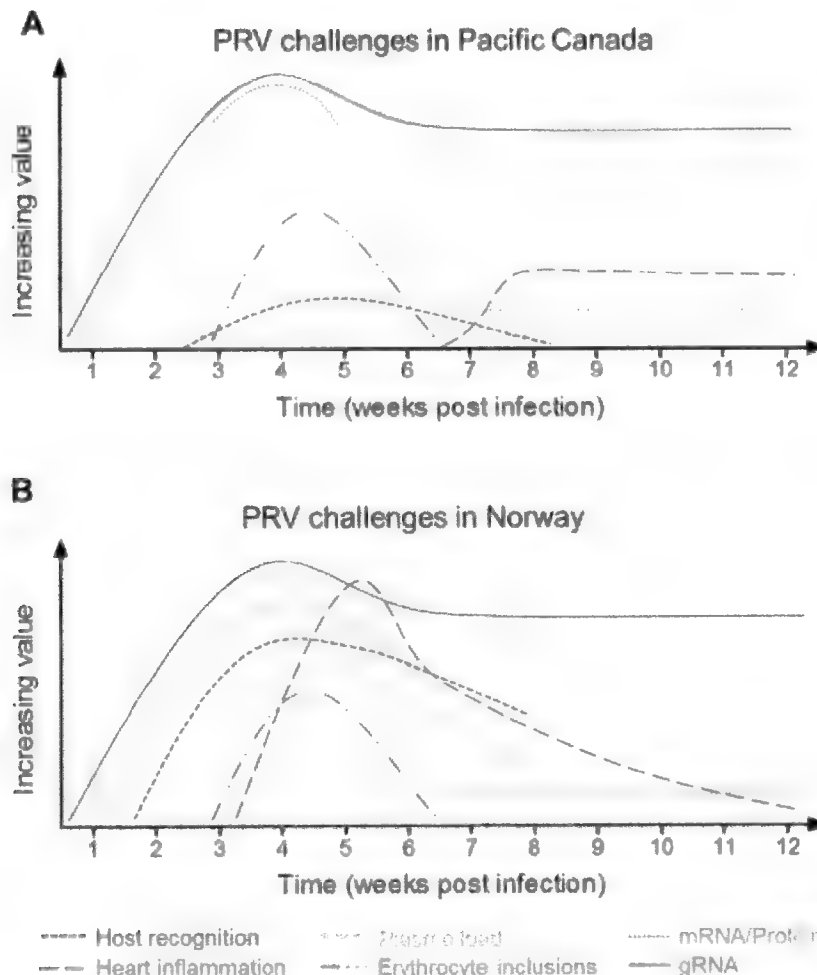
The phenotypic characteristics of both Canadian and Norwegian PRV during infection have at least three potentially significant dissimilarities (Fig. 12). First, the inability to detect Pacific Canada PRV (either 16-005ND or 16-011D) in the blood plasma of infected fish during our challenges is in stark contrast with PRV loads reported in plasma of infected fish following challenge with Norwegian PRV, where sometimes substantial PRV plasma loads are generated for a period lasting at least six weeks (2 to ≥8 following infection)<sup>1, 41</sup>. Second, there is a considerable difference in scale regarding host recognition of PRV. Although direct comparisons between Canadian and Norwegian studies are limited because Canadian studies have assessed gene expression relative to time-matched controls whereas Norwegian studies have referenced expression to time zero, it is nevertheless conspicuous that mean systemic and heart-specific antiviral responses increased no more than fivefold in our study whereas in Norwegian challenges these genes increased 10-50 fold in the blood<sup>1, 47</sup> and more than 100 fold in the heart<sup>21</sup>. The comparative lack of antiviral response to Pacific Canada PRV compared to Norwegian PRV is further supported by the relative protection PRV has afforded to fish challenged with a secondary virus (IHNV) in Norway<sup>48</sup> but not in Pacific Canada<sup>28</sup>. Lastly, in addition to the discrepancies concerning the severity of heart inflammation, the timing of PRV associated heart inflammation is also different between challenges conducted with PRV from these two countries. Specifically, by either injection or cohabitation exposure of PRV, heart inflammation (prevalence and severity) in Norwegian studies consistently begins around the time of peak systemic PRV load, reaches high severity 1-2 weeks later, and thereafter diminishes<sup>1, 20</sup>. In contrast, increased prevalence of heart inflammation in our challenge trials did not occur until approximately 4 weeks after peak PRV systemic loads were reached and maintained high prevalence (although not severity) for the remaining 6-7 weeks of the trial.

Taken together, these findings suggest that phenotypic differences in infection kinetics between PRV from Norway and Pacific Canada stem at least in part from genetic variation between the two regional variants. Although a specific virulence factor (or set of factors) remains unclear, we hypothesize that the increased prevalence of Norwegian PRV outside red blood cells (i.e. in the plasma) might help to explain the heightened recognition of PRV by Norwegian fish and that this recognition could contribute to the elevated inflammatory immune responses

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observed. Because the S1 segment is responsible for the spread of mammalian orthoreovirus<sup>49</sup> and also has high genetic diversity between Norwegian and Canadian PRV<sup>14</sup>, it presents a likely candidate for further investigation as a virulence factor for PRV. However, the involvement of cytotoxic T-cells and robust type-II interferon response generated in heart tissues of HSML diseased Mowi Strain Atlantic salmon in Norway<sup>21</sup> almost certainly enhances or even causes the observed tissue damage. PRV from Pacific Canada also elicits a specific cytotoxic T-cell response in heart tissues of Pacific-adapted Mowi-McConnell Atlantic salmon, but to far less of a degree<sup>50</sup>. This presents the possibility that HSML might result (or be enhanced) by a host-associated hypersensitivity heightened in the Norwegian-adapted Mowi strain of Atlantic salmon farmed in Norway relative to the Pacific-adapted Mowi-McConnell strain farmed in Pacific Canada. This hypothesis is supported by the development of an HSML resistant strain of Mowi Atlantic salmon in Norway that is resistant to disease but not PRV infection<sup>51, 52</sup>. Ultimately we believe that both host and virus specific factors likely play a role in the development of HSML, and further investigations are needed to pinpoint the mechanisms responsible for generating this disease.



**Figure 12: Contrast summary for trends in PRV phenotypic infection dynamics between Norway and Canada laboratory challenge of Atlantic salmon.** In comparing the present challenge trials conducted in (A) Pacific Canada with results from similar challenge trials conducted in (B) Norway<sup>1, 21, 41, 47</sup>, the kinetics of viral RNA, protein (indirectly measured in Canada by mRNA or directly measured in Norway by florescent antibody staining) and erythrocytic inclusion body formation follow a similar pattern. However, the kinetics regarding the plasma load of PRV, transcriptional induction of genes involved in host recognition of virus by Atlantic salmon, as well as the severity and timing of PRV associated heart inflammation appear notably discrete between the two countries. For these comparisons, timing is presented relative to first signs of infection and not necessarily the initiation of a challenge since detectable infections take longer to develop following cohabitation than by i.p. injection. It should

also be noted that no data are yet available with regard to the transcriptional host responses or plasma PRV loads beyond 8 weeks post infection in Norwegian based studies and thus their late stage kinetics are unknown and not presented. Lastly, Y-axis scale is not intended to be interpreted as absolute.

The kinetics of North American PRV in Atlantic salmon as observed in this study indicates three distinct phases of infection: early, peak, and persistent infection. In the first (early) phase of infection which lasts 2-3 weeks, initial replication and dissemination of the virus into the blood cells of the host occurs. During this period, viral 'factories' (inclusions) were not observed in erythrocytes and there seemed to be no systemic recognition of virus by host cells. This indicates that the virus might be replicating by an alternative process relative to later time points or it might be initially infecting a different cell type. Mammalian orthoreoviruses first infect epithelial cells of the small intestine or lung prior to hematogenous dissemination<sup>53</sup>, and the recent detection of PRV in intestinal enterocytes<sup>8</sup> indicates that a similar course of infection might be followed by PRV. Regardless, it is expected that this early replicative phase dictates the overall severity of infection<sup>54</sup>, as it likely accounts for how many erythrocytes ultimately become infected. This is supported by the discrepancy in total virus production following challenge with PRV 16-005ND compared to 16-011D in this study, where an initial lag in 16-011D replication appeared to be the major difference between otherwise identical replication dynamics within the two challenges. What caused the poor primary infection rate following challenge with PRV 16-011D compared to 16-005ND is unclear; however, the lack of PRV transmission via fish cohabitation at this early stage of infection suggests that whatever cell type the virus is infecting during this period, it is not likely being shed into the environment to a high degree.

In the second (peak) phase of infection that lasts 2-3 weeks, substantial PRV replication within erythrocytes occurs along with the formation of cytoplasmic viral inclusions. The large spherical inclusions are similar to those previously reported in Norway<sup>41, 47, 55</sup> as well as to those that develop during mammalian reovirus infection of well-established cell lines<sup>56</sup>. The highest systemic loads of PRV RNA that occur during this period appeared sufficient in some instances to initiate mild systemic host recognition of virus. As host recognition was only observed following 16-005ND challenge and not following PRV 16-011D challenge in this study, we speculate that this recognition might have been load dependent since PRV 16-005ND reached higher transcriptional quantities during this period. From previous cohabitation challenges, it is also concluded that substantial shedding of virus occurs at this time<sup>13</sup>.

In the third (persistent) phase of infection, viral inclusions within erythrocytes disappear. No systemic host recognition of virus occurs, and a marked reduction in viral protein production (as measured by mRNA concentration) occurs even though large quantities of genomic PRV material remain associated with the erythrocyte cell fraction. This supports the hypothesis that infectious PRV is retained in the cytoplasm of infected erythrocytes but is in a reduced or non-replicative state. The ability to recapitulate infectious replication of PRV from this late stage of infection was readily accomplished by injecting lysed blood cell material into naïve fish which generated comparable temporal infection dynamics to virus that had been harvested during the peak phase of infection (Fig. 6). However, poor viral transmission occurred via cohabitation during this late infectious stage, suggesting natural shedding of virus might be minimal during persistent infections and may even cease entirely over time. This is supported by the reduced rates of cohabitation infection at 15 wpc compared to 9 wpc in this study, and the inability to transmit virus via cohabitation after 45 to 63 wpc as demonstrated previously<sup>13</sup>. Nevertheless, infectious virus was still present in the blood of infected fish in this study for at least 15 wpc and it is presumed that the rather substantial quantities of RNA detected in blood at 63 wpc by Garver *et al.*<sup>13</sup> also represented at least a moderate amount of infectious PRV particles.

For this study, we focused mainly on PRV L1 RNA to monitor viral loads in accordance with a number of



610 previously published works<sup>13, 16, 28, 57, 58, 59</sup>. However, some studies have targeted alternate segments of the PRV  
611 genome for relative quantification. Specifically, Haatveit *et al.* identified differential temporal expression patterns of  
612 PRV S1 compared to M2 and M3 during the persistent phase of infection in a Norwegian challenge trial, where the  
613 relative quantities of S1 were approximately 9 Ct less than M2 and M3 (a >500 fold theoretical reduction)<sup>47</sup>. In our  
614 study, the relative quantity of all 10 genomic segments was comparable during all three phases of viral replication  
615 with less than fourfold proportional divergence between any two given segments (Fig. 5). This indicates that for the  
616 Pacific Canada PRV we tested, any of the 10 segments could be used interchangeably to estimate the PRV  
617 abundance within a sample at any given time. In Norwegian based studies, temporal expression patterns of L3<sup>20</sup> as  
618 well as M2 and M3<sup>47</sup> appear similar to what has been observed here; however, the considerable reduction in S1  
619 expression observed by Haatveit *et al.*<sup>47</sup> provides yet further support for altered viral kinetics between Norwegian  
620 and Pacific Canadian strains of PRV that may stem from alterations in the S1 segment of the genome.

621 In addition to exploring the expression patterns of all 10 PRV genomic segments, we also developed a new  
622 and relatively simple technique for differentiating the amount of PRV single-stranded mRNA from double-stranded  
623 genomic material. It is likely that this technique can be applied in the detection of RNA from any virus with a dsRNA  
624 genome which might have expanded application in the laboratory exploration of dsRNA viruses. In specific context  
625 to PRV, we observed that the quantity of mRNA (and by implication the quantity of new viral proteins being made)  
626 became significantly reduced during the final persistent phase of infection. This is in line with previous observations  
627 of  $\lambda 1$ ,  $\mu 1$ ,  $\sigma 1$  and  $\sigma 3$  PRV protein production as noted in late stage infections of Atlantic salmon in Norway<sup>47</sup>. We  
628 also observed relatively minor but statistically significant proportional differences of mRNA quantities for individual  
629 PRV genomic segments which varied depending on the phase of infection. However, the implications for this latter  
630 variability is unknown and could be inconsequential given that almost all variation was encompassed within a  
631 twofold deviation from complete proportional equality.

632 One important aspect of the host immune response to PRV that was not addressed in this study was the  
633 putative development of antibodies. In Norway, host Atlantic salmon have been demonstrated to generate  
634 detectable antibodies specific to PRV  $\mu 1c$  and  $\mu NS$  in the plasma that began approximately two weeks after peak  
635 systemic PRV loads were reached and were maintained at detectable levels until the end of the study one month  
636 later<sup>60</sup>. It is unknown as to whether antibodies were generated against PRV in our current study; however, given the  
637 high viral loads, relative ease for horizontal transmission, and mild but significant antiviral recognition observed  
638 during peak PRV infection in this study, we speculate that at least some PRV specific antibodies were likely  
639 produced. If so, there may be future potential in exploring the avirulent characteristics of the Pacific Canada PRV  
640 used here to potentially protect against Norwegian isolates of PRV that have been associated with causing HSML,  
641 particularly since both formalin killed PRV and DNA plasmids expressing PRV  $\mu NS$  have demonstrated at least  
642 partial protection against HSML in Norway<sup>22, 61</sup>.

643 In conclusion, although we were able to identify an HSML-like disease state in farmed Atlantic salmon of  
644 Pacific Canada, this regionally rare condition could not be effectively transmitted via injection of PRV infected blood  
645 material into naïve fish as has been accomplished in Norway. This study also revealed genotypic and phenotypic  
646 differences between PRV from Pacific Canada compared to what has been reported from PRV challenge trials in  
647 Norway. These differences suggest that virus and/or host specific factors are likely needed for the development of  
648 HSML in farmed Atlantic salmon and that currently PRV infections of Atlantic salmon in Pacific Canada are of low  
649 virulence. Further research is needed to determine the potential cause or causes for HSML-like lesions in Pacific

Canada. Also, because interactions between Atlantic salmon, PRV, and the environmental conditions of Pacific Canada do not appear conducive to HSMI development, the low virulence associated with Pacific Canada PRV provides a useful model for comparative studies to investigate the requirements for initiating PRV-associated disease and exploring possible protection mechanisms against PRV associated disease such as HSMI.

## Methods

**Fish source and husbandry.** Atlantic salmon for the challenge studies were sourced from a single commercial freshwater hatchery on Vancouver Island, British Columbia and brought to the Pacific Biologic Station (PBS) in Nanaimo, British Columbia, Canada. Pre-transport screening of 20 fish via qPCR was negative for PRV. Tissue homogenates from 90 fish in the hatchery were negative for culturable agents; no cytopathic effect was observed on CHSE or EPC cell lines prior to transport. Fish were of a Pacific-adapted Mowi-McConnell strain of Atlantic salmon with at least 30 years isolation from the originating European stocks<sup>27</sup>. Once at PBS, fish were maintained in UV treated municipal freshwater ( $10^{\circ} \pm 1^{\circ}\text{C}$ ) for 3 months prior to smoltification to undiluted sand-filtered UV treated seawater ( $11^{\circ} \pm 1^{\circ}\text{C}$ , 32 ppt). A natural photoperiod was used during culture and fish were fed dry pellets (EWOS) at 1-2% body weight per day prior to challenge. This cohort was used as a source of PRV negative control inoculum, provided the naïve recipients for the primary PRV 16-005ND and 16-011D i.p. injection challenges, and also provided the naïve recipients for the subsequent i.p. and cohabitation viral passage experiments using PRV 16-005ND. During all challenge trials, fish were maintained on undiluted UV treated seawater ( $11^{\circ} \pm 1^{\circ}\text{C}$ , 32 ppt) and fed a ration of EWOS pellets at 1% body weight per day.

## PRV detection and quantification.

**PRV (L1) detection by TaqMan qPCR.** PRV nucleic acid was detected from blood, heart and plasma samples by real-time qPCR with slight modification to previously described methods<sup>13, 28</sup>. In summary, total RNA was extracted from 100  $\mu\text{L}$  blood, 100  $\mu\text{L}$  plasma, or ~50mg heart tissue in TRIzol Reagent (Life Technologies) as per manufacturer's instructions using 5 mm steel beads and TissueLyser II (Qiagen) which operated for 2 min at 25 Hz. A portion of eluted RNA (1.0  $\mu\text{g}$ ) was denatured for 5 min at  $95^{\circ}\text{C}$ , immediately cooled to  $4^{\circ}\text{C}$ , and reverse-transcribed using a High Capacity cDNA Reverse Transcription kit (Life Technologies) following the manufacturer's instructions. Resulting cDNA was used directly as template for qPCR analysis in a StepOne-Plus real-time detection system (Applied Biosystems) using primers and TaqMan probe targeting the L1 fragment of the PRV genome<sup>28</sup>. Each reaction contained 400 nM primers and 300 nM TaqMan probe, 1X TaqMan Universal Master Mix and 1  $\mu\text{L}$  cDNA template within each 15  $\mu\text{L}$  reaction. Cycling conditions included an initial incubation of  $95^{\circ}\text{C}$  for 10 min followed by 40 cycles of  $95^{\circ}\text{C}$  for 10 s and  $60^{\circ}\text{C}$  for 20 s. Samples were assayed in duplicate and were considered positive if both technical replicates reported a Ct value < 40 cycles. Absolute PRV quantification was determined in each instance by serial dilution of a 482 bp double-stranded DNA gBLOCK fragment (Integrated DNA Technologies) consisting of sequence targeted by the qPCR primer and probe<sup>13</sup>. A seven-step 10-fold dilution series of the gBLOCK fragment spanning a dynamic range of  $10$ - $10^7$  target copies per reaction was incorporated in duplicate into each run. The limit for accurate quantitative assessment using this technique was calculated to be between 10-50 copies with a limit of detection of 1-3 copies per reaction determined as previously described<sup>59</sup>.



*PRV (all segment) detection by SYBR<sup>®</sup> green qPCR.* Primers specific to each of the 10 PRV genome segments were designed using Primer3<sup>60</sup> within the Geneious 9.1.7 software platform<sup>61</sup> to homogenous protein-coding regions within four published PRV genomes previously identified from Pacific Canada: VT06062012-358<sup>42</sup>; BCJ19943\_13<sup>14</sup>; and WSKFH12\_14<sup>14</sup> (Supplement 1). A portion (1 µL) of cDNA generated using a High Capacity Reverse Transcription kit from Trizol extracted RNA as described above was added to duplicate 15 µL qPCR reactions containing 500 nM forward and reverse primers and 1x final concentration of Power SYBR<sup>®</sup> green PCR master mix (ThermoFisher) in molecular grade water. Reactions were analyzed in a StepOne-Plus real-time qPCR detection system (Applied Biosystems) with an initial polymerase activation at 95 °C for 10 min followed by 40 cycles 5 s at 95°C, 20 s at 60°C, and 10 s at 72°C with fluorescence measured at the end of the 72°C step. Melt curve analyses were performed to ensure amplification specificity and a five-step fourfold dilution series of cDNA prepared from the blood of a highly infected individual (sample #35; 16-005ND4 at 4 wpc) was performed in duplicate on each run to estimate relative quantity and amplification efficiency.

*ssRNA PRV qPCR detection and validation.* All TaqMan or SYBR qPCR analyses designed to exclusively amplify the ssRNA and not dsRNA component of the targeted PRV segment were performed as described above with the exception that RNA was heated to 80°C for 1 min rather than 95°C for 5 min prior to cDNA synthesis. Total RNA from PRV 16-005ND infected fish at 10 wpc was used to validate this differential detection of PRV ssRNA by exposing total RNA (2 µg) to either no enzyme, 2 U Pure Link<sup>™</sup> RNase A (ThermoFisher Scientific), or 2 U RNase A in the presence of 0.5 M sodium chloride which selectively protects dsRNA but not ssRNA from RNase A degradation<sup>32</sup>. Following incubation at 25° C for 45 min, RNA (if remaining) was recovered using RNeasy MinElute Cleanup Kit (Qiagen) as per manufacturer's instructions. Recovered RNA was heated to 55°C for 5 min, 80°C for 1 min, or 95°C for 5 min, immediately cooled to 4°C and reverse transcribed using a High Capacity cDNA Reverse Transcription kit (Life Technologies) following the manufacturer's guidelines. Resulting cDNA was used directly as template for PCR analysis in a StepOne-Plus detection system with 500 nM PRV L1 (this study) or Atlantic salmon β-actin<sup>13</sup> forward and reverse primers and 1X Power SYBR<sup>®</sup> Green PCR master mix (ThermoFisher). Cycling conditions were performed as described above but were ended after 30-cycles prior to fluorescence saturation in samples for which product was amplified. A portion (5 µL) of product was then visualized by UV excitation on a 3% agarose Tris-borate-EDTA (TBE) gel containing 0.5x SYBR<sup>®</sup> Safe DNA stain after 45 min migration at 120 volts in 1X TBE running buffer (ThermoFisher).

**PRV sequencing.** Library construction, sequencing services and bio-informatics support was provided by the Canadian Centre for Computational Genomics and Génome Québec Innovation Centre, Montréal, Canada. RNA extracted from the blood of four fish were selected for library construction and RNA-seq analysis – two from fish challenged with PRV 16-005ND (sample numbers 161 and 165) and two from fish challenged with PRV 16-011D (sample numbers 167 and 171) collected at 7 wpc (Fig. 2A). A portion (10 µg) of the total RNA extracted from each samples was purified using 2 U of DNase I (Life technologies) at 37°C for 45 min followed by RNeasy MinElute Cleanup (Qiagen) as per manufacturer's instructions. RNA quality was visualized on a 1% bleach denaturing gel<sup>62</sup> and ensured to have a Bioanalyzer (Agilent) RNA Integrity Number (RIN) > 9. RNA sequencing was performed using half of one lane (8 libraries per lane) of an Illumina<sup>®</sup> HiSeq 2500 (Illumina Inc.) platform using a NEBNext<sup>®</sup> rRNA Depletion Kit (Human/Mous/Rat) with read lengths of 125 bp. Base calls were made using the Illumina

CASAVA pipeline encoded in Phred 33. The two libraries generated for both 16-005ND and 16-011D challenges were pooled and *de novo* transcript assembly was performed on combined reads (2 libraries per assembly) following the pipeline described by Haas *et al.*<sup>63</sup> based on the Trinity assembly software suite<sup>64</sup>. In brief, reads were trimmed using Trimmomatic software<sup>62</sup> from the 3' end with a minimal Phred score of 30 and a minimum length of 32 bp. A normalized metric of reads was generated using Trinity normalization utility and surviving paired reads were assembled using the Trinity assembler<sup>63</sup>. Putative assembled transcripts were aligned against the NCBI Viral Genomes Resource database<sup>63</sup> using the blastn program from the NCBI BLAST family. Transcripts which aligned to PRV sequences with an Expect value (E value) less than  $e^{-100}$  were aligned by segment using Geneious 9.1.7 to report the consensus sequence for the longest positive-sense genomic strand in each instance.

**PRV and HSMI sampling during natural infection.** Moribund or recently deceased net-pen farmed Atlantic salmon were collected as part of a pretransfer fish health audit conducted by the Fisheries and Oceans Canada, Aquaculture Management Division. With relevance to this study, skeletal muscle and heart tissues were preserved in 10% neutral buffered formalin and processed as previously described<sup>5</sup>. On July 5<sup>th</sup>, 2016, an audit of 40 fish (36 moribund/dead; 4 live) was conducted at a net-pen farm site in the Johnstone Strait of BC, for which skeletal muscle and heart tissues were collected for histology. The site had 12 operational pens; each containing between 30-50 thousand fish per pen with fish weighing approximately 500g each. On July 29<sup>th</sup> and August 7<sup>th</sup>, 2016, samples of heart and skeletal muscle from 5 and 6 moribund/dead fish, respectively, were also collected for histology by a private veterinarian working for the source farm.

A final sampling was conducted August 19<sup>th</sup>, 2016 by MP and KG, in which 20 fish were sampled specifically for this study – six fish were moribund/dead, eight were from the general population (apparently healthy), and six were non-performers of low body condition. Blood (0.5-3 mL) was collected from the caudal vein of each fish using 22 gauge needle and 3 mL syringe. A 100 µL subsample was immediately frozen in liquid nitrogen and used for PRV L1 TaqMan qPCR screening as described above. Remaining blood was divided into 1 mL aliquots and stored at -80°C for use in generating challenge inoculating material described below. Heart and skeletal muscle from each fish was preserved in 10% neutral buffered formalin for histopathologic evaluation.

#### **PRV challenge of Atlantic salmon by i.p. injection.**

**Inoculum preparation.** PRV 16-005ND was initially sourced from a cohort of healthy Atlantic salmon in March of 2016 held at a commercial freshwater Atlantic salmon rearing facility on Vancouver Island, Canada. The facility had no history of HSMI and PRV material collected from this site in 2013 had failed to generate HSMI in previous laboratory challenge trials<sup>13, 28</sup>. PRV infected blood of hatchery fish (~25g) which had been frozen at -80°C prior to use was passed through ~30 g British Columbia Mowi-McConnell Atlantic salmon held in brackish water (15ppt) for three weeks at 11°C, passed again through ~50g Atlantic salmon held in seawater (32 ppt) for three weeks at 11°C, and passed a third time in ~55 g Atlantic salmon held in seawater for four weeks at 11°C prior to final collection. In each instance, blood from three infected fish was pooled, diluted 1:10 in Hank's balanced salt solution (HBSS), sonicated on ice for 80 s in 10 s bursts with 30 s rests using a Branson Digital Sonifier 250/450 at 20% amplitude, clarified via centrifugation at 2,000 × g for 5 min at 4°C, and administered to naïve fish by 100 µL intra-peritoneal injection. Following the third passage, blood from three fish was pooled and inoculate prepared as for previous passages.

PRV 16-011D was sourced from the blood of three fish (#3, 5, 15) collected at the net-pen farm site in which fish had HSML-like lesions on August 19<sup>th</sup>, 2016 (Supplement 1). The blood from these fish was pooled, diluted 1:10 in HBSS, sonified and clarified as described for 16-005ND. An identical preparation of sonified and clarified diluted blood was prepared from a pooled sample of three PRV-free individuals sourced from the cohort of fish used for all subsequent challenge trials to provide vehicular control inoculate.

*Intra-peritoneal injection and monitoring.* Atlantic salmon (~70 g each) were anesthetized in an aqueous solution of Tricaine methanesulfonate (0.05 g/L) and given a 200 µl intra-peritoneal injection of either PRV 16-005ND inoculum, PRV 16-011D inoculum, or PRV-free vehicular control inoculum (90 fish per treatment). Fish were placed in treatment-specific 850 L circular tanks (one tank per inoculum) supplied with 30 L/min 11°C (± 1°C) UV treated seawater (32 ppt). Temperature, dissolved oxygen, feeding performance, and morbidity/mortality were monitored daily throughout the challenge trials (Supplement 1).

*PRV and HSML associated sampling.* Fish were anesthetized in an aqueous solution of Tricaine methanesulfonate (0.05 g/L), and blood and tissue samples were collected from six fish per treatment tank at 7 day intervals through 15 wpc. Blood (~2 mL) was collected using a 22 ga needle and 3 mL syringe. A 100 µL aliquot was immediately frozen in liquid nitrogen and subsequently used for PRV screening and gene expression analysis by qPCR as described above. Approximately 10 µL was transferred to a sodium-heparin treated Fisherbrand™ micro-hematocrit tube and spun at 15,000 × g for 10 min for hematocrit determination and a second 10 µL was smeared on a glass microscope for cytoplasmic inclusion body visualization. Slides were air dried, fixed in 100% methanol for 5 min, and stained with pinacyanol chloride<sup>41</sup>. Remaining blood was transferred to a heparinized vacutainer and spun at 2,000 × g for 5 min at 4°C. Plasma (100 µL) was transferred to a clean 2 mL microtube and immediately frozen at -80°C prior to PRV qPCR screening as described above. An approximate 200 mg section of red/white skeletal muscle was excised from the left lateral line at approximately the mid-body and preserved in 10% neutral buffered formalin for histopathology. Hearts were bisected longitudinally and one half preserved in 10% neutral buffered formalin for histopathology while the other half was immediately frozen in liquid nitrogen for qPCR analyses. Tissues in 10% NBF were fixed for 24-48 hours, transferred to 70% isopropanol and paraffin embedded following standard methods. Sections 3 µm thick were transferred to glass slides and stained routinely with haematoxylin and eosin for light microscopy<sup>5</sup>. Photomicrographs were optimized for illumination and color balance<sup>64</sup>. To ensure inter-sample consistency in lesion scoring as well as consistency relative to published PRV studies from Norway, approximately 10% of slides (29/338) were examined by both principal pathologists (GDM and HNS) and a subset of 60 slides was also sent to a third reviewing pathologist (RJ) in Norway for further examination (Supplement 1). All histopathology was conducted blind to PRV exposure status and scores provided by the other pathologists.

At 10 wpc PRV 16-005ND, blood of three fish was separated into plasma, leukocyte, and erythrocyte components. Plasma was collected following centrifugation of the heparinized blood at 2,000 × g for 5 min at 4°C. The cell pellet was suspended to an original blood volume using HBSS and layered over a 34%/51% isotonic percoll discontinuous gradient and centrifuged at 800 × g for 20 min at 4°C which was allowed to come to rest without the use of the centrifuge breaking system. Peripheral blood leukocytes were harvested from the 34%/51% interphase and erythrocytes from the pelleted material below the 51% layer. Cells were washed twice with HBSS, verified for >99% purity via hemocytometer, and suspended at a final concentration of 10e<sup>7</sup> cells per 100 µL HBSS

which was used for PRV qPCR screening.

**Passage of PRV via cohabitation or i.p. injection.** PRV 16-005ND inoculate not used in the injection challenge was thawed and administered to three sets of 15 fish (~150 g per fish) by intra-peritoneal injection, 200 µL per injection, under MS-222 anesthesia. Fish were cultured in 850 L tanks as above with the exception that the volume of 11°C seawater was reduced to 250 L and supplied with a flow of 15 L per min. After a period of 1, 9, or 15 wpc, an equal number (n=15) naïve cohabitants (with clipped adipose fins) were introduced to each to the three tanks. Blood samples were collected from six of both injected (shedder) and introduced (sentinel) fish after 4 and 8 weeks of cohabitation and screened for PRV L1 transcripts as described above.

**Comparative analyses.** Trinity assembled PRV segments were concatenated and compared to two previously published PRV genomes (B5690 V3621 by Jukes-Cantor phylogenetic relationship analysis using Geneious software. The relative quantities (scaled to the minimum value) of PRV L1 following differential pre-amplification denaturation were compared at 2, 4, and 10 wpc for both PRV 16-005ND and 16-011D challenged groups by one-way ANOVA and Tukey post-test of log-transformed data. The proportional change of PRV ssRNA (relative to total PRV RNA) was as assessed over time in both PRV 16-005ND and 16-011D challenged groups by one-way ANOVA and Dunnett's multiple comparison post-test of arcsin-transformed values. The relative quantity (scaled to the minimum value) of each PRV 16-005ND RNA segment as well as the single-stranded mRNA proportion of each segment was assessed at 2, 4, and 10 wpc by two-way ANOVA with Bonferroni posttests follow log-(relative quantity) and arcsin-(proportional quantity) transformation. The contributing proportion to total PRV RNA and total single-stranded mRNA of each segment were considered independent and dependent to time, respectively, by two-way ANOVA. Thus, total PRV segment expression was pooled from 2, 4, and 10 wpc and compared by one-way ANOVA and Tukey's post-hoc test of arcsin transformed values and single-stranded mRNA proportional expression was compared separately at each time point by one-way ANOVA with Dunnett's multiple comparison post-test relative to L1. The trend in hematocrit of both PRV 16-005ND and 16-011D challenged fish were compared to controls by two-way ANOVA with Bonferroni posttests of arcsine transformed values. This was similarly applied to comparing the quantity of erythrocyte cytoplasmic inclusion bodies observed in PRV 16-005ND versus 16-011D infected fish. All gene expression data was normalized to  $\beta$ -actin transcription. Normalized quantities were scaled to the minimum value for each gene prior to analysis of log-transformed data by two-way ANOVA with Bonferroni posttests. Heart and skeletal muscle histopathology inflammation scores for PRV 16-005ND and 16-011D infected fish estimated at each time point throughout challenge was compared to control fish by Mann Whitney U tests without multiple comparison adjustment.

**Author contributions.** MP and KG conceived and designed the study. MP conducted sampling, performed data analysis and interpretation, and drafted the manuscript. GM and HS performed histopathological examination. All authors read, contributed to, and approved the final manuscript.

**Competing interests.** The authors declare they have no competing interests.

**Data availability.** Data presented in this manuscript are provided in supplement 1, available through NCBI SRA

850 SRP145317, or NCBI GenBank accessions MH347359 – MH347378.

851

852 **Ethics statement.** All work with animals was performed in strict accordance with the recommendations set out by  
853 the Canadian Council on Animal Care (CCAC) guide to the care and use of experimental animals and all live animal  
854 protocols were approved by the Pacific region animal care committee (animal use protocol number: 16–013). All  
855 fish handling was performed under Aquacalm™ (Syndel Laboratories Ltd.) or tricaine methanesulfonate (MS222)  
856 anesthesia.

857

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Article

# Piscine Orthoreovirus 3 Is Not the Causative Pathogen of Proliferative Darkening Syndrome (PDS) of Brown Trout (*Salmo trutta fario*)

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**Abstract:** The proliferative darkening syndrome (PDS) is a lethal disease of brown trout (*Salmo trutta fario*) which occurs in several alpine Bavarian limestone rivers. Because mortality can reach 100%, PDS is a serious threat for affected fish populations. Recently, Kuehn and colleagues reported that a high throughput RNA sequencing approach identified a piscine orthoreovirus (PRV) as a causative agent of PDS. We investigated samples from PDS-affected fish obtained from two exposure experiments performed at the river Iller in 2008 and 2009. Using a RT-qPCR and a well-established next-generation RNA sequencing pipeline for pathogen detection, PRV-specific RNA was not detectable in PDS fish from 2009. In contrast, PRV RNA was readily detectable in several organs from diseased fish in 2008. However, similar virus loads were detectable in the control fish which were not exposed to Iller water and did not show any signs of the disease. Therefore, we conclude that PRV is not the causative agent of PDS of brown trout in the rhithral region of alpine Bavarian limestone rivers. The abovementioned study by Kuehn used only samples from the exposure experiment from 2008 and detected a subclinical PRV bystander infection. Work is ongoing to identify the causative agent of PDS.

**Keywords:** proliferative darkening syndrome; black trout syndrome; piscine orthoreovirus; orthoreovirus; brown trout; *Salmo trutta fario*; next generation sequencing; RT-qPCR

## 1. Introduction

The brown trout (*Salmo trutta fario*) is a predatory fish of the family *Salmonidae*. Its range of distribution covers nearly the entirety of Europe, and its natural habitats are fast flowing, cool,

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**To:** Miller-Saunders, Kristi  
**Subject:** FW: Draft PRV SAR For Your Review  
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My comments on the SAR are enclosed.

Kristi Miller

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**Cc:** Olivier, Gilles; Craig Stephen; Parsons, Jay

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Hello all,

My comments:

Line 31: "consequences to Fraser River Sockeye Salmon abundance and diversity..." At some point in the document it would be good to clarify that we were assessing all Fraser River sockeye stocks as a whole (i.e. not at the CU basis), and what the definition of "negligible" is as it relates to the degree of loss of abundance/diversity

Line 56: it might be worth making explicit that the detection of PRV-1 genetic material does not indicate viable, infective PRV-1 virus.

Line 117: Same comment as for line 56. Perhaps here would be a better place to make clear that the inability to culture the virus necessitates bioassays to determine the presence/absence of "live" infective PRV. Detection of genetic material is not synonymous with infectivity.

Line 243: "...beginning of June..."

Line 381: "...exposure time and dose required to result in a PRV-1 infection..."

Line 412: suggest clarifying that "salmon" refers to both farmed Pacific (Chinook) salmon and Atlantic salmon.

Line 467-469: I am not clear what is being said here, particularly "...including need for minimal standards of diagnostic tools." Suggest rewording to clarify.

Cheers,

Zac

**From:** Niccolò Vendramin [mailto:niven@aqu.dtu.dk]

**Sent:** March-13-19 9:43 AM

**To:** Gagne, Nellie <Nellie.Gagne@dfo-mpo.gc.ca>; Farrell, Anthony <tony.farrell@ubc.ca>; Weber, Lily <Lily.Weber@dfo-mpo.gc.ca>; Burgetz, Ingrid <Ingrid.Burgetz@dfo-mpo.gc.ca>; Waddington, Zac <Zac.Waddington@dfo-mpo.gc.ca>; Struthers, Alistair <Alistair.Struthers@dfo-mpo.gc.ca>; 'Nathalie.N.Bruneau@inspection.gc.ca' <Nathalie.N.Bruneau@inspection.gc.ca>; 'Myron.Roth@gov.bc.ca' <Myron.Roth@gov.bc.ca>; [REDACTED]; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; [REDACTED]; 'espen.rimstad@nmbu.no' <espen.rimstad@nmbu.no>; 'mark.powell@hi.no' <mark.powell@hi.no>; 'iagardner@upei.ca' <iagardner@upei.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Polinski, Mark <Mark.Polinski@dfo-mpo.gc.ca>; Mimeault, Caroline <Caroline.Mimeault@dfo-mpo.gc.ca>; Holt, Kendra <Kendra.Holt@dfo-mpo.gc.ca>; Johnson, Stewart <Stewart.Johnson@dfo-mpo.gc.ca>; Jones, Simon <Simon.Jones@dfo-mpo.gc.ca>; [REDACTED];

[REDACTED]; 'Gary.Marty@gov.bc.ca' <Gary.Marty@gov.bc.ca>; [REDACTED];

[REDACTED]; Boily, France <France.Boily@dfo-mpo.gc.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>

**Cc:** Olivier, Gilles <Gilles.Olivier@dfo-mpo.gc.ca>; Craig Stephen <cstephen@cwahc-rcsf.ca>; Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>

**Subject:** RE: Draft PRV SAR For Your Review

Dear all,

Thanks for the valuable effort in compiling all information from the meeting.

A couple of inputs from here.

s.19(1)

1) Line 456 "Undertake an assessment of factors influencing risk of importing exotic strains of PRV into BC". Exotic strains for PRV is a definition that is too open/unprecise. Could it be amended as "avoid introduction of PRV-1 isolates with genetic markers increasing the risk of developing HSMI" and "avoid introduction of PRV genogroups other than PRV-1"

2) The following reference should be included in the reference list.

- Di Cicco E, Ferguson HW, Kaukinen KH, Schulze AD, Li S, Tabata A, Günther OP, Mordecai G, Suttle CA, and Miller KM. 2018. The same strain of Piscine orthoreovirus (PRV-1) is involved in the development of different, but related, diseases in Atlantic and Pacific Salmon in British Columbia. FACETS 3: 599–641. doi:10.1139/facets-2018-0008" this should be included.

3) if it can be of any help, we got recently published the paper of PRV-3 causing heart pathology in Rainbow trout.

- Vendramin N, Kannimuthu D, Olsen AB, Cuenca A, Teige LH, Wessel Ø, Iburg TM, Dahle MK, Rimstad E, Olesen NJ (2019) Piscine orthoreovirus subtype 3 (PRV-3) causes heart inflammation in rainbow trout (*Oncorhynchus mykiss*). Vet Res 50:14 . doi: 10.1186/s13567-019-0632-4

<https://veterinaryresearch.biomedcentral.com/articles/10.1186/s13567-019-0632-4>

Best regards

Niccolò and Espen

**From:** Gagne, Nellie [<mailto:Nellie.Gagne@dfo-mpo.gc.ca>]

**Sent:** 12. marts 2019 20:48

**To:** Farrell, Anthony; Weber, Lily; Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair;

'Nathalie.N.Bruneau@inspection.gc.ca'; 'Myron.Roth@gov.bc.ca'; [REDACTED] Miller-Saunders, Kristi;

[REDACTED] 'espen.rimstad@nmbu.no'; Niccolò Vendramin; 'mark.powell@hi.no';

'iagardner@upei.ca'; Garver, Kyle; Polinski, Mark; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon;

[REDACTED] 'Gary.Marty@gov.bc.ca'; [REDACTED] Boily, France; Garver, Kyle

**Cc:** Olivier, Gilles; Craig Stephen; Parsons, Jay

**Subject:** RE: Draft PRV SAR For Your Review

A few comments/suggestion in addition:

Ln 48-50: the first sentence is about variation of virulence due to the virus itself, but the second could be about the genetic of the fish... is the first sentence correct? Or it should be ... variation in the virulence among strains of PRV-1 and/or among Atlantic Salmon of different origins.

Ln 108: due to PRV...

Ln 109: released ? (instead of transferred)

Ln 128 and 156: in conjunction

Ln 132: or any severe skeletal... (was there any skeletal inflammation, even mild?)

Ln 163: is it preliminary or the data is now analysed ?

Ln 172: cut the sentence (too long)

Ln 358 and 265 and 428: is minimal = throughout the paper I think the "negligible" label as in fig 3 is used, whereas here it says minimal

Ln 395: not consensus to change.. (grammar or rewording required)

All the best,

Nellie

**From:** Farrell, Anthony <[tony.farrell@ubc.ca](mailto:tony.farrell@ubc.ca)>

**Sent:** March-08-19 2:59 PM

**To:** Weber, Lily <[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca)>; Burgetz, Ingrid <[Ingrid.Burgetz@dfo-mpo.gc.ca](mailto:Ingrid.Burgetz@dfo-mpo.gc.ca)>; Waddington, Zac <[Zac.Waddington@dfo-mpo.gc.ca](mailto:Zac.Waddington@dfo-mpo.gc.ca)>; Struthers, Alistair <[Alistair.Struthers@dfo-mpo.gc.ca](mailto:Alistair.Struthers@dfo-mpo.gc.ca)>; Gagne, Nellie

<Nellie.Gagne@dfo-mpo.gc.ca>; 'Nathalie.N.Bruneau@inspection.gc.ca' <Nathalie.N.Bruneau@inspection.gc.ca>;  
'Myron.Roth@gov.bc.ca' <Myron.Roth@gov.bc.ca>; [REDACTED] Miller-  
Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; [REDACTED]  
[REDACTED] 'espen.rimstad@nmbu.no' <espen.rimstad@nmbu.no>; 'niven@vet.dtu.dk'  
<niven@vet.dtu.dk>; 'mark.powell@hi.no' <mark.powell@hi.no>; 'iagardner@upei.ca' <iagardner@upei.ca>; Garver,  
Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Polinski, Mark <Mark.Polinski@dfo-mpo.gc.ca>; Mimeault, Caroline  
<Caroline.Mimeault@dfo-mpo.gc.ca>; Holt, Kendra <Kendra.Holt@dfo-mpo.gc.ca>; Johnson, Stewart  
<Stewart.Johnson@dfo-mpo.gc.ca>; Jones, Simon <Simon.Jones@dfo-mpo.gc.ca>; [REDACTED]  
[REDACTED]  
[REDACTED]; 'Gary.Marty@gov.bc.ca' <Gary.Marty@gov.bc.ca>; [REDACTED]  
[REDACTED]; Boily, France <France.Boily@dfo-mpo.gc.ca>; Garver,  
Kyle <Kyle.Garver@dfo-mpo.gc.ca>  
Cc: Olivier, Gilles <Gilles.Olivier@dfo-mpo.gc.ca>; Craig Stephen <cstephen@cwahc-rscf.ca>; Parsons, Jay  
<Jay.Parsons@dfo-mpo.gc.ca>  
Subject: Re: Draft PRV SAR For Your Review

All

Unless I comment otherwise below, I am accepting any suggested edits on the version sent to me.

The following specific minor comments are for clarity, conciseness and correctness.

All the best

Tony

L28: A 'single' uncertainty cannot have a range. Perhaps you mean the "contributing uncertainties" ...  
L33 was = were; "were discussed. Expert participants voiced different opinions on the ....

L36 This piece is awkwardly constructed and points 1 and 2 have an unbalance sentence structure. Try...

The main uncertainties were (1) the likelihood of infection of wild salmon PRV-1 from infected Atlantic salmon farms, and (2) the consequences to Sockeye Salmon. In the former case, uncertainty exists because of the lack of data to estimate the concentration of PRV-1 from infected Atlantic salmon farms, the exposure duration required for infection to occur and the minimum infectious dose for adult and juvenile Sockeye salmon. For the later, uncertainty exists in applicability laboratory studies to estimate consequences.

L47 the = the current

L53 replace with "... but with typically considerably lower..."

L59 replace with "... trails with juvenile...". See line 69

L60 "... of a disease ..."

L63 fresh water = freshwater

s.19(1)

L70 reword to be more precise and concise

moderate lesions, without any fish mortality, clinical signs or anaemia.

L72 reword to be more precise and concise

In four independent laboratory challenge trials with juvenile Sockeye Salmon, high viral loads of PRV-1 were generated without any fish mortality, clinical signs or anaemia. The interpretation of the histopathology results (i.e., lesions) was inconclusive.

L78 can not = cannot

L121 "...more frequent detection in Coho ..."

L141 "... some farms in Norway..."

L267 "... Salmon and confirmed only transient..."

L468 "... including the need ..."

**From:** "Weber, Lily" <[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca)>

**Date:** Thursday, March 7, 2019 at 5:43 AM

**To:** "Burgetz, Ingrid" <[Ingrid.Burgetz@dfo-mpo.gc.ca](mailto:Ingrid.Burgetz@dfo-mpo.gc.ca)>, "Waddington, Zac" <[Zac.Waddington@dfo-mpo.gc.ca](mailto:Zac.Waddington@dfo-mpo.gc.ca)>, "Struthers, Alistair" <[Alistair.Struthers@dfo-mpo.gc.ca](mailto:Alistair.Struthers@dfo-mpo.gc.ca)>, "Gagne, Nellie" <[Nellie.Gagne@dfo-mpo.gc.ca](mailto:Nellie.Gagne@dfo-mpo.gc.ca)>, "Nathalie.N.Bruneau@inspection.gc.ca" <[Nathalie.N.Bruneau@inspection.gc.ca](mailto:Nathalie.N.Bruneau@inspection.gc.ca)>, Myron Roth <[Myron.Roth@gov.bc.ca](mailto:Myron.Roth@gov.bc.ca)>, [REDACTED], Kristi Miller-Saunders <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>, [REDACTED], [REDACTED] <[espen.rimstad@nmbu.no](mailto:espen.rimstad@nmbu.no)>, "niven@vet.dtu.dk" <[niven@vet.dtu.dk](mailto:niven@vet.dtu.dk)>, "mark.powell@hi.no" <[mark.powell@hi.no](mailto:mark.powell@hi.no)>, "iagardner@upei.ca" <[iagardner@upei.ca](mailto:iagardner@upei.ca)>, Kyle Garver <[Kyle.Garver@dfo-mpo.gc.ca](mailto:Kyle.Garver@dfo-mpo.gc.ca)>, "Polinski, Mark" <[Mark.Polinski@dfo-mpo.gc.ca](mailto:Mark.Polinski@dfo-mpo.gc.ca)>, "Weber, Lily" <[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca)>, "Mimeault, Caroline" <[Caroline.Mimeault@dfo-mpo.gc.ca](mailto:Caroline.Mimeault@dfo-mpo.gc.ca)>, "Holt, Kendra" <[Kendra.Holt@dfo-mpo.gc.ca](mailto:Kendra.Holt@dfo-mpo.gc.ca)>, "Johnson, Stewart" <[Stewart.Johnson@dfo-mpo.gc.ca](mailto:Stewart.Johnson@dfo-mpo.gc.ca)>, Simon Jones <[Simon.Jones@dfo-mpo.gc.ca](mailto:Simon.Jones@dfo-mpo.gc.ca)>, [REDACTED], [REDACTED], "Farrell, Anthony" <[tony.farrell@ubc.ca](mailto:tony.farrell@ubc.ca)>, [REDACTED], "Gary.Marty@gov.bc.ca" <[Gary.Marty@gov.bc.ca](mailto:Gary.Marty@gov.bc.ca)>, [REDACTED], [REDACTED], "Boily, France" <[France.Boily@dfo-mpo.gc.ca](mailto:France.Boily@dfo-mpo.gc.ca)>, Kyle Garver <[Kyle.Garver@dfo-mpo.gc.ca](mailto:Kyle.Garver@dfo-mpo.gc.ca)>  
**Cc:** "Olivier, Gilles" <[Gilles.Olivier@dfo-mpo.gc.ca](mailto:Gilles.Olivier@dfo-mpo.gc.ca)>, Craig Stephen <[cstephen@cwbc-rcsf.ca](mailto:cstephen@cwbc-rcsf.ca)>, "Parsons, Jay" <[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca)>  
**Subject:** Draft PRV SAR For Your Review

Dear Participants of the CSAS peer review on the risk to Fraser River Sockeye Salmon for PRV from Atlantic salmon farms in the Discover Islands area:

On behalf of the co-chairs (Gilles Olivier and Craig Stephen), attached please find the draft version of the Science Advisory Report (SAR) for the PRV risk assessment that we reviewed in January 2019.

We are seeking your comments and approval of this document. Recall that at the meeting we had agreed to the summary bullets, Recommendations and Other Considerations, so please focus your review on the other parts of the report including the Introduction, Analysis, Sources of Uncertainty, Conclusions, etc. to assess if there are any factual errors or omissions or other comments. However, you will note that there are a few changes to the summary bullets, recommendations and other considerations which were made to improve clarity and grammar. Please let us know if you agree with these changes.

We would appreciate receiving your comments by **Friday, March 22, 2019**. After which we will review all input, finalise the report, seek the chairs' and internal approvals, and format for posting on the DFO web site.

Thank you.

**Lily Weber**

Science Advisor, Aquaculture, Biotechnology and Aquatic Animal Health Science Branch  
Fisheries and Oceans Canada / Government of Canada

[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca) / Tel: [REDACTED]

Conseillère scientifique, Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques  
Pêches et Océans Canada / Gouvernement du Canada

[Lily.Weber@dfo-mpo.gc.ca](mailto:Lily.Weber@dfo-mpo.gc.ca) / Tel: [REDACTED]



Government  
of Canada

Gouvernement  
du Canada

s.16(2)(c)

**Canada**

No further information has been removed or severed from this page



## Miller-Saunders, Kristi

---

**From:** Miller-Saunders, Kristi  
**Sent:** April-03-19 9:21 AM  
**To:** Miller-Saunders, Kristi  
**Subject:** FW: List of SC potential reviewers and participants\_PRV\_KM.xlsx  
**Attachments:** List of SC potential reviewers and participants\_PRV\_KM.xlsx

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

**From:** Miller-Saunders, Kristi  
**Sent:** November-29-18 4:07 PM  
**To:** Malcolm, Gabrielle <Gabrielle.Malcolm@dfo-mpo.gc.ca>; Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>  
**Subject:** List of SC potential reviewers and participants\_PRV\_KM.xlsx

Here are my recommendations.

Hope it is helpful,  
Kristi

## Proposed Participants

**DRAFT**

**\*If you have additional suggestions for potential participants, please add their names and affiliations in the empty cells below**

Need a better balance of PRV-related disease experts around the world, especially those who study Pacific salmon  
Note that half of the fish health expertise collaborates with Garver on PRV research

Name	Expertise	Role	Affiliation	Location	Comments
	Fish Health and genomics	Participant	BC CAHS	Campbell River, BC	
Gary Marty	Fish Health and Pathology	Participant	Animal Health Centre, BC Ministry of Agriculture	Abbotsford, BC	
Tony Farrell	Fish Biology and Physiology	Participant	UBC	Vancouver, BC	
Ian Gardner	Fish Health, epidemiology and risk assessment	Participant	UPEI AVC	Charlottetown, PEI	
Maureen Purcell	Fish health, virologist	Participant	USGS	Washington	
Mark Powell	Fish Health, aquaculture and risk assessment	Participant	IMR Norway	Norway	
Øystein Wessel	Virologist/ Veterinary science	Participant	NMBU	Oslo, Norway	
Espen Rimstad	Virologist/Veterinary science	Participant	NMBU	Oslo, Norway	Senior virologist to replace Øystein
Emiliano DiCicco	Pathologist/Veterinary science	Participant	Pacific Salmon Foundation/ PBS	Nanaimo, BC	
	Fish health and epidemiology	Participant	Veterinary Consultant		
	Salmon biology/ecology/populations	Participant	Kintama	Nanaimo, BC	
	Fish Health and aquaculture	Participant	Grieg Seafood	Campbell River, BC	
Ted Meyers	Fish health	Participant	Alaska Department of Fish and Game	Juneau	
Hugh Ferguson	Pathologist/Veterinary science	Participant	Saint Georges University	Grenada	World-class pathologist who works in Canada, Europe and Chile; HSMI and heart disease expert
	Salmon stock assessment, ecology, management	Participant	Pacific Salmon Foundation	Vancouver, BC	

Where are the First Nations?

s.19(1)

**Pages 447 to / à 448  
are duplicates of  
sont des duplicatas des  
pages 14 to / à 15**

## Proposed Reviewers

**DRAFT**

\*If you have additional suggestions for potential reviewers, please add their names and affiliations in the empty cells provided under each working paper category

Name	Expertise	Role	Working papers 1. PRV and cardiopathy characterization 2. PRV risk assessment	Affiliation	Location	Comments
Ian Gardner	Fish Health, epidemiology and risk assessment	Reviewer	PRV Risk assessment	UPEI AVC	Charlottetown, PEI	
Mark Powell	Fish Health, aquaculture and risk assessment	Reviewer	PRV Risk assessment	IMR Norway	Norway	part of study PRV-infection reduces the tolerance to hypoxic stress in Atlantic
		Reviewer	PRV Risk assessment			
		Reviewer	PRV Risk assessment			
Øystein Wassel	Virologist/ Veterinary science	Reviewer	PRV and cardiopathy	NMBU	Oslo, Norway	
Maureen Purcell	Fish health, virologist	Reviewer	PRV and cardiopathy	USGS	Washington	
Mark Powell	Fish Health, aquaculture and risk assessment	Reviewer	PRV and cardiopathy	IMR Norway	Norway	part of study PRV-infection reduces the tolerance to hypoxic stress in Atlantic
Hugh Ferguson	Pathologist, veterinary science/PRV disease worldwide	Reviewer	PRV and cardiopathy	Saint Georges University	Grenada	Among the first pathologists to describe HSMI/involved in PRV-related disease studies worldwide

NOTE:  
Nobody identified who studies PRV-related diseases in Pacific Salmon

s.19(1)  
s.21(1)(b)

## Miller-Saunders, Kristi

---

**From:** Miller-Saunders, Kristi  
**Sent:** April-03-19 9:44 AM  
**To:** Miller-Saunders, Kristi  
**Subject:** FW: ADVANCED COPY: Peer Review concludes Piscine Orthoreovirus transfer from Atlantic salmon farms poses minimal risk to wild Fraser River sockeye/ L'examen par les pairs conclut que le risque de transfert de l'orthoréovirus pisciaire des élevages...

Kristi Miller-Saunders, PhD  
Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

s.21(1)(a)

s.21(1)(b)

-----Original Message-----

**From:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Sent:** February-07-19 3:58 PM  
**To:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Subject:** RE: ADVANCED COPY: Peer Review concludes Piscine Orthoreovirus transfer from Atlantic salmon farms poses minimal risk to wild Fraser River sockeye/ L'examen par les pairs conclut que le risque de transfert de l'orthoréovirus pisciaire des élevages...

---

**From:** MacDougall, Lesley  
**Sent:** February 7, 2019 2:36 PM  
**To:** Garver, Kyle; Miller-Saunders, Kristi; Higgins, Mark; Jones, Simon  
**Cc:** Rainer, Michelle; Geiger, Karen  
**Subject:** FW: ADVANCED COPY: Peer Review concludes Piscine Orthoreovirus transfer from Atlantic salmon farms poses minimal risk to wild Fraser River sockeye/ L'examen par les pairs conclut que le risque de transfert de l'orthoréovirus pisciaire des élevages...

Hi all;

A media technical briefing was held this morning on the findings of the PRV peer review: there were a number of difficult questions, there are a number of participants who are rather upset that we went ahead with the briefing without releasing the documents, so I want to be sure you're aware in case you start to get calls.

To give a bit of background – although we haven't done technical briefings after peer reviews out here before, it's not uncommon (e.g. in NFLD) to have a media briefing right after a meeting to just announce the meeting took place, provide a quick overview of the questions being considered, the scope, and the key areas of consensus. It used to be standard practice to have one after every national CSAS meeting but that has fallen away in the last number of years. In this case it was felt that it was important to provide some messaging regarding the findings that were agreed on during

the meeting, and that outweighed the risk associated with being out ahead of when the final documents would be ready. In retrospect, our intentions were good – to be proactive, and improve transparency – but the timing ended up working against us in that it could be perceived as reactionary given the federal court decision, even though planning had started for it prior to the completion of the peer review meeting itself. Some of the questions were more related to the decision than to the peer review, understandably.

You may have reporters contacting you in the next few days in follow up, so I want you to both be aware of the briefing and of the materials that were circulated to help if you've got questions coming in.

Lesley

From: Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>

Sent: February-07-19 12:00 PM

To: MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>

Subject: FW: ADVANCED COPY: Peer Review concludes Piscine Orthoreovirus transfer from Atlantic salmon farms poses minimal risk to wild Fraser River sockeye/ L'examen par les pairs conclut que le risque de transfert de l'orthoréovirus pisciaire des élevages...

Carmel

Carmel Lowe, Ph.D.

Regional Director Science | Directrice régionale des sciences Fisheries and Oceans Canada | Pêches et Océans Canada

Pacific Biological Station | Station biologique du Pacifique

3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7 Carmel.Lowe@dfo-mpo.gc.ca<mailto:Carmel.Lowe@dfo-mpo.gc.ca>

Telephone | Téléphone 250-756-7177

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Government of Canada | Gouvernement du Canada

From: Girouard, Louise <Louise.Girouard@dfo-mpo.gc.ca<mailto:Louise.Girouard@dfo-mpo.gc.ca>>

Sent: February 7, 2019 11:48 AM

To: Thomson, Andrew <Andrew.Thomson@dfo-mpo.gc.ca<mailto:Andrew.Thomson@dfo-mpo.gc.ca>>; Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca<mailto:Carmel.Lowe@dfo-mpo.gc.ca>>; Reid, Rebecca <Rebecca.Reid@dfo-mpo.gc.ca<mailto:Rebecca.Reid@dfo-mpo.gc.ca>>

Cc: Antcliffe, Bonnie <Bonnie.Antcliffe@dfo-mpo.gc.ca<mailto:Bonnie.Antcliffe@dfo-mpo.gc.ca>>; Webb, Allison <Allison.Webb@dfo-mpo.gc.ca<mailto:Allison.Webb@dfo-mpo.gc.ca>>; Bate, Dan <Dan.Bate@dfo-mpo.gc.ca<mailto:Dan.Bate@dfo-mpo.gc.ca>>; Rainer, Michelle <Michelle.Rainer@dfo-mpo.gc.ca<mailto:Michelle.Rainer@dfo-mpo.gc.ca>>

Subject: FW: ADVANCED COPY: Peer Review concludes Piscine Orthoreovirus transfer from Atlantic salmon farms poses minimal risk to wild Fraser River sockeye/ L'examen par les pairs conclut que le risque de transfert de l'orthoréovirus pisciaire des élevages...

Please see final NR and Backgrounder to be issued shortly - the call has started.

L

From: ComApproval / Approbation (DFO/MPO) <ComApproval/Approbation.XNCR@dfo-mpo.gc.ca<mailto:ComApproval/Approbation.XNCR@dfo-mpo.gc.ca>>

Sent: Thursday, February 7, 2019 11:40 AM

To: McElhone, Kathryn <Kathryn.Mcelhone@dfo-mpo.gc.ca<mailto:Kathryn.Mcelhone@dfo-mpo.gc.ca>>; Quinn, Caroline <Caroline.Quinn@dfo-mpo.gc.ca<mailto:Caroline.Quinn@dfo-mpo.gc.ca>>; Jenkins, Phil <Phil.Jenkins@dfo-mpo.gc.ca<mailto:Phil.Jenkins@dfo-mpo.gc.ca>>; Seguin, Natalie <Natalie.Seguin@dfo-mpo.gc.ca<mailto:Natalie.Seguin@dfo-mpo.gc.ca>>; ComApproval / Approbation (DFO/MPO)

<ComApproval/Approbation.XNCR@dfo-mpo.gc.ca<mailto:ComApproval/Approbation.XNCR@dfo-mpo.gc.ca>>; NCR Multimedia RCN (DFO/MPO) <Multimedia.XNCR@dfo-mpo.gc.ca<mailto:Multimedia.XNCR@dfo-mpo.gc.ca>>; Morris, Christina <Christina.Morris@dfo-mpo.gc.ca<mailto:Christina.Morris@dfo-mpo.gc.ca>>  
Subject: ADVANCED COPY: Peer Review concludes Piscine Orthoreovirus transfer from Atlantic salmon farms poses minimal risk to wild Fraser River sockeye/ L'examen par les pairs conclut que le risque de transfert de l'orthoréovirus pisciaire des élevages...

News release and Backgrounder / Communiqué et Fiche d'information The attached products will be issued shortly. / Les produits ci-joint sera émis sous peu.  
Peer Review concludes Piscine Orthoreovirus transfer from Atlantic salmon farms poses minimal risk to wild Fraser River sockeye/ L'examen par les pairs conclut que le risque de transfert de l'orthoréovirus pisciaire des élevages de saumon de l'Atlantique au saumon rouge sauvage du fleuve Fraser est minime

Distribution : National EN & FR Comprehensive

## Miller-Saunders, Kristi

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**From:** DiCicco, Emiliano  
**Sent:** April-05-19 10:30 AM  
**To:** Miller-Saunders, Kristi  
**Subject:** Agenda of the collaboration of the coast meeting in May  
**Attachments:** 4b2fd737-4511-4216-aaf4-64d3fa56563d.pdf

Agenda of the collaboration of the coast meeting in May... it really looks like DFO and C. are trying to say that PRV is in the marine environment... and therefore industry can't do anything about that...

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## Collaborations on the Coast Aquaculture Canada 2019 Agenda

Time	Title	Speaker
8:15 – 8:30	Introduction	Moderator - [REDACTED]
8:30 – 9:30	Keynote "Seafood for the Future Program"	[REDACTED] Aquarium of the Pacific
9:30 - 10:00	Nutrition Break	
10:00 – 10:10	Reconvene	
Wild and Farm-Raised Salmon Interactions		
10:10 – 10:30	Salish Sea Marine Survival Program - Outcomes	[REDACTED] Pacific Salmon Foundation
10:30 – 10:50	Acoustic Tagging – interactions of wild salmon with fish farms in Discovery Islands	[REDACTED] Kintama Research Ltd.
10:50 – 11:10	Monitoring stomach content of farm-raised salmon in BC	Kerra Shaw, DFO
11:10 – 11:30	Questions / Discussion	Moderator – [REDACTED]
Fish Health Investigations		
11:30 – 11:50	Pathogen Baseline Survey – BC Coastal Region	Beibei Jia, UPEI
11:50 – 12:10	Investigations in gill health	Simon Jones, DFO
12:10 – 1:20	Lunch - provided	

\*AGENDA SUBJECT TO CHANGE

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## Collaborations on the Coast Aquaculture Canada 2019 Agenda

1:20 – 1:30	Reconvene	
1:30 – 1:50	PRV – fitness of sockeye salmon	Tony Farrell, UBC (or post-doc)
1:40 – 2:10	PRV – environmental reserves	Mark Polinski, DFO
2:10 – 2:30	PRV and resident wild species around salmon farms	BC CAHS
2:30 – 2:50	Questions / Discussion	Moderator –
2:50 – 3:20	Nutrition Break	
3:20 – 3:30	Reconvene	
Salmon Ecology		
3:30 – 3:50	Gulf of Alaska Expedition (title to come)	Chrys Neville, DFO
Sea Lice		
3:50 – 4:10	Comparative susceptibility to sea lice among salmon species in the North Pacific	Simon Jones, DFO
4:10 – 4:20	Investigations of perch health profile in consideration as a cleaner fish in BC	Stewart Johnson, DFO
4:20 – 4:30	Discussion / Wrap Up	Moderator –

s.19(1)

\*AGENDA SUBJECT TO CHANGE

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** April-05-19 11:59 AM  
**To:** Polinski, Mark  
**Cc:** [REDACTED] Miller-Saunders, Kristi; [REDACTED] DiCicco, Emiliano;  
Minister / Ministre (DFO/MPO); Popham, Lana; Bob Chamberlin  
**Subject:** Re: Two Question on Paper on PRV in Nature

Dear Dr. Mark Polinski:

A month has passed without a response from you.

Please refer us to the research you rely on in your citations that HSMI has been found in PRV-free salmon and provide the studies reporting morbidity in farm salmon with heart lesions is "uncommon."

Thank you,

[REDACTED]

> On Mar 14, 2019, at 1:57 PM, [REDACTED] wrote:

>

> Dear Dr. Mark Polinski:

>

> Congratulations on your publication in the high impact journal Nature.

>

> I am writing for clarification on your statement that HSMI has been diagnosed in BC salmon in absence of PRV.

>

> In your section titled Preface concerning the diagnosis of HSMI; you rely on two papers in making the statement that HSMI has been reported in farm salmon in absence of PRV: "... if HSMI diagnosis is based solely on histopathological heart lesions which can occur in the absence of PRV..." The papers you appear to cite are Marty et al (2015) and Di Cicco et al (2018).

>

> This is an important conclusion, an outlier to other literature on PRV in Atlantic salmon, and it is a "preface" to the work you report on in your paper.

>

> However, Di Cicco et al (2018) say that they suspect that the one fish reported the farm audit data with heart lesions in absence of PRV "was not an HSMI fish".

> Similarly, Marty et al (2015), state: "None of the fish in our study had microscopic lesions diagnostic for HSMI." They noted heart damage, but suggest it was due to *Loma salmonae* infection, and did not view it as HSMI.

>

> Neither of these papers support the conclusion that HSMI occurs in absence of PRV.

>

> Similarly in 2016, yourself and your co-authors, reported that western North America is "a region now considered endemic for PRV but without manifestation of HSMI" (Garver et al 2016). This is a sweeping statement that includes all the farm salmon health audits, in all years and the private reports by Dr. Marty's lab to industry. This suggest that prior to 2016 HSMI was never seen in BC.

>

s.19(1)

> However, four months after Garver et al (2016) stated that HSMI does not manifest in BC, Dr Marty appears to reverse his position in an internal email that he did find HSMI-like lesions in BC farm salmon beginning in at least 2008 (attached).

>

> When I asked Dr. Marty to describe the difference between HSMI and HSMI-like he said there was "no difference" (attached). Excuse me if I have this wrong, but this suggests that when Garver et al (2016) and Marty et al (2014) were published, HSMI actually had been observed in BC, but this observation is not reported and none of you report HSMI in a PRV-free Atlantic salmon.

>

> Going back to your paper in Nature, you are very specific that you are referring to the organ damage caused by HSMI: "Consequently, if HSMI diagnosis is based solely on histopathological heart lesions which can occur in the absence of PRV, then PRV cannot be assumed to be the causative agent of the disease, but rather one of multiple stand-alone or synergistic putative factors."

>

> My confusion stems from papers published by the authors of your paper that state HSMI has not been found in BC and so I don't know where to find reporting that HSMI has been diagnosed without PRV.

>

> It is confusing - HSMI lesions were recognized in BC farm salmon in 2008 by the Province of BC farm salmon audits, but in 2014 and 2016 it was reported that HSMI has not been observed in BC, and now it is stated that HSMI does occur in BC and sometimes without PRV, citing a paper that states that HSMI does not occur in BC.

>

> I hope you can see my cause for confusion.

>

> Another note of concern where you state that morbidity in farm salmon with heart lesions is "uncommon" you cite several unpublished works from 1990-1992, a time period when the industry was predominantly farming Pacific salmon, which are not known to develop HSMI. So looking for morbidity in Pacific salmon with heart lesions, does not appear to inform on morbidity in Atlantic salmon with HSMI. In my surveys, morbidity and PRV are common in BC farm salmon today (see attached). Can you supply the reports you cite in 23-26?

>

> So two questions:

>

> • Which studies report HSMI in absence of PRV in Atlantic salmon?

> • Can you supply the unpublished reports cited as 23-26?

>

> Thank you for your consideration of this important matter. I have copied others working on PRV in BC.

>

>

>

>

>

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>

>

> <2017-0400HSMIAdvice [redacted] Interim.pdf>

>

> <Finners.jpg>

> <ATIP A2016-203 HSMI recognized.pdf>

>

s.19(1)

## Miller-Saunders, Kristi

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**From:** DiCicco, Emiliano  
**Sent:** April-05-19 12:26 PM  
**To:** Andrew Bateman  
**Cc:** Miller-Saunders, Kristi  
**Subject:** RE: histology sample selection

Thanks Andrew.

I was looking at the first spreadsheet... please consider that we do have the ISH probe for CTV, but we don't have any for te\_mar, pa\_pse and Mitov. We just got ISH probes for Nidov, pspv and ukRNAv in the lab (as Kristi requested), and we do have also ISH probes for pa\_min, pa\_ther, ce\_sha, and a few other viruses. I can still do morphological histo (i.e. standard H&E staining) on all those samples, though.

ISH for ASCV and PRV won't be an issue as we have the probes for both of them. [REDACTED]

Talk you soon,

Emiliano

**From:** Andrew Bateman <[REDACTED]>  
**Sent:** April-04-19 9:12 PM  
**To:** DiCicco, Emiliano <Emiliano.DiCicco@dfo-mpo.gc.ca>  
**Subject:** histology sample selection

Hi Emiliano,

I finally sat down to go through the aquaculture longitudinal samples to see what might be the best for in-situ work for the agents we discussed with Kristi. Attached are a couple spreadsheets. The first identifies four individuals for several agents, spread across live and dead fish, and in most cases across farms. Mitov individuals were all dead, since it only shows up in dead fish, and in-situ work would hopefully tell us whether the virus is in host cells or not. The second spreadsheet identifies six individuals that are either high/high or high/low in ASCV/PRV, in an effort to get at a possible connection there. Again, individuals are both alive and dead.

Have a look and see what you think.

Talk soon,

Andrew

s.19(1)

s.20(1)(b)

s.21(1)(b)

## Miller-Saunders, Kristi

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**From:** DiCicco, Emiliano  
**Sent:** April-05-19 2:28 PM  
**To:** Miller-Saunders, Kristi  
**Cc:** Schulze, Angela; Kaukinen, Karia; Li, Shaorong; Ming, Tobi; Tabata, Amy; Gideon Mordecai; Suttle, Curtis  
**Subject:** new phylogenetic study on PRV... from Kibenge and C.

FYI...

<https://virologyj.biomedcentral.com/articles/10.1186/s12985-019-1148-2?fbclid=IwAR2ImVaY0UvYRFBnJGdUBW9M51MGoguvhZXKQWAUFVEtyLXP-QMLuYEgAk>

No information has been removed or severed from this page

## Miller-Saunders, Kristi

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**From:** Higgins, Mark  
**Sent:** April-09-19 10:17 AM  
**To:** MacDougall, Lesley; Withler, Ruth; Holmes, John; Miller-Saunders, Kristi; Kennedy, Eddy  
**Subject:** RE: ADM I&T April 2019.ppt

Thanks for sending this along Lesley. A couple of comments so far, while the crux of the court decision was for transfers of fish from land based hatcheries to marine operation, and marine to marine transfers, it also has implications to SEP releases from freshwater hatcheries to the marine environment. I don't get a sense that this has been incorporated into the deck. Implications to SEP releases should be spelled out in this.

I am also having trouble with the statement "Prevalence of disease agent is below propagation threshold for receiving environment". In the case of PRV, even if the population to be transferred is clean (negative for PRV), our studies have shown that after 100-200 days in sea water, near 100% fish will become infected with the virus. So if all fish are going to end up with PRV in the end, the propagation is more a function of the environment, not the transfer.

There also seems to be an arrow missing from that box in the 'no' direction (i.e. Prevalence of disease agent below... has a yes arrow down to 'aggregates of concern box', but what if it is not below the propagation threshold).

Mark.

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** April-08-19 4:20 PM  
**To:** Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Withler, Ruth <Ruth.Withler@dfo-mpo.gc.ca>; Holmes, John <John.Holmes@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Kennedy, Eddy <Eddy.Kennedy@dfo-mpo.gc.ca>  
**Subject:** FW: ADM I&T April 2019.ppt

Hi all;

To keep you in the loop with the development of a response to the recent court decision, Jay Parsons will be presenting this to brief senior management to give them an idea of the current thinking for how to triage and articulate the decisions made.

Bear in mind this is still draft; I've noted that slide 10 identifying operational support needed should also clearly note that commenting on whether a disease agent is 'below propagation threshold for receiving environment' will be challenging as we move to pathogens and agents we know little about.

As drafts become available I'll share them with you –  
Lesley

**From:** Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>  
**Sent:** April-08-19 2:26 PM  
**To:** Webb, Allison <Allison.Webb@dfo-mpo.gc.ca>; MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Cc:** Burgetz, Ingrid <Ingrid.Burgetz@dfo-mpo.gc.ca>  
**Subject:** ADM I&T April 2019.ppt

Allison, Leslie:

Please see attached the deck we have put together to brief senior management. We could use this for the AMD meeting tomorrow if you think it would be appropriate?

Also, I am proposing to use a similar version to brief Science Management on Thursday.

Any comments/suggestions are welcome.

Thanks, Jay

No information has been removed or severed from this page



## Miller-Saunders, Kristi

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**From:** DiCicco, Emiliano  
**Sent:** April-10-19 12:49 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** RE: Away

Thanks Kristi.

[REDACTED]

Just to keep you in the loop, Christoph and I are going to Tofino next week to sample Tranquil creek and Bedwell. We basically prepared everything, and we are ready to go. We plan to do dissections on 0+ and 1+coho and 0+ chinook with button scar (i.e. egg sack already absorbed) ... in order to provide the training for the people over there. I will also go on the Nimpkish in May (before heading to Italy) to do a day of training for their field sampling in concert with industry (both sealice and pathogen screening). I had a phone call with the FN's layer yesterday... and we have to plan to work along [REDACTED] and potentially Andrew to put together a research proposal to present Carmel in order to access the lab for the interim FN testing.

On the Lab side, I just printed the list of samples to be used to run the ISH on nidovirus, UkrRNA and reov. Unfortunately, the samples with the best loads belong to AMD, and we don't have them! So, I put together a list of the remaining ones... and I'll work with Tobi to find them. I will also shadow her on the work for the histo sample preparation. I also have a list of "interesting" samples to run histo and ISH from Andrew, but unfortunately we don't have a probe for most of the agents he's interested on (mostly bacteria, parasite and Mitov). I will try to find the ones for CTV, ASCV and PRV for sure, though... as we have probes for them. I also put the scores of the PRV amplification challenge on Excel... here in attachment is the file. Finally, as you must have seen from previous emails... I worked on Hugh's paper pictures... and sent them to him. We should be ok on that, now.

If there is something else you need me to work on... just let me know.

Emiliano

s.19(1)

**From:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Sent:** April-10-19 12:22 PM  
**To:** DiCicco, Emiliano <Emiliano.DiCicco@dfo-mpo.gc.ca>  
**Subject:** FW: Away

Sorry, forgot to send to you

---

**From:** Miller-Saunders, Kristi  
**Sent:** April 10, 2019 12:17 PM  
**To:** Kaukinen, Karia; Schulze, Angela; Ming, Tobi; Tabata, Amy; Li, Shaorong; Christoph Deeg; Sutherland, Ben; [REDACTED] Andrew Bateman; Amy Teffer; [REDACTED] gideon mordecai  
**Cc:** Withler, Ruth; Beacham, Terry; Scott Hinch; 'Suttle, Curtis'; Glenn Crossin; [REDACTED] McLeod, Patricia; MacDougall, Lesley; Lowe, Carmel  
**Subject:** Away

[REDACTED]

I would appreciate being kept in the loop, and I do still check emails, but I would very much appreciate it if you guys can work together on decisions that need to be made and discussions on manuscripts, field sampling etc... I don't have a lot of bandwidth at present, and may not get things back that need to be read at the pace anyone would like. I can still take the odd call to deal with emerging issues.

Scott, you should probably remove me from any student committees/comprehensives/etc for the next month [REDACTED]

Ruth and Terry, one of you will need to act for me. Andrew, if you could take the lead with our epidemiology team, I would appreciate it. Karia and Amy, could you please lead the Quinsam sampling? Ben, sorry I did not get back in time on your proposal, [REDACTED] and after all, you are the lead! I am excited by the potential however. Terry, I am going to contact [REDACTED] and ask that you represent our lab for at least the GBC F2F. I know that he was concerned that they would have questions about the Fit Chips, so perhaps I can go over that with you in more detail, and you can get more information on progress and potential on the MinION from Chris and Ben. [REDACTED] if you could connect with GBC and get an extension on our report due end of April, I would appreciate it.

I know there are other things I am supposed to be doing that I am not thinking of, so you can contact me if you have questions. My cell number is [REDACTED]

Thanks all,

Kristi

s.16(2)(c)

s.19(1)

## Miller-Saunders, Kristi

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**From:** Withler, Ruth  
**Sent:** April-10-19 3:54 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** RE: Away

Hi,  
I'm going to be acting while you are away this month so let me know if you need to be looped in on anything that you are not getting. As you can infer from the email exchange between Corino and Lesley, she stepped in to deal with the sampling issues at Quinsam and has got SEP to agree to develop an ongoing sampling plan with you when you get back. The PRV response and the Framework development are continuing, not sure that there will be much opportunity for input but will forward anything that comes around. [REDACTED] a lot of section stuff likely only to be sent to me by actors; I'll forward all relevant stuff.

[REDACTED]  
Ruth

**From:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Sent:** April-10-19 12:17 PM  
**To:** Kaukinen, Karia <Karia.Kaukinen@dfo-mpo.gc.ca>; Schulze, Angela <Angela.Schulze@dfo-mpo.gc.ca>; Ming, Tobi <Tobi.Ming@dfo-mpo.gc.ca>; Tabata, Amy <Amy.Tabata@dfo-mpo.gc.ca>; Li, Shaorong <Shaorong.Li@dfo-mpo.gc.ca>; Christoph Deeg <[REDACTED]>; Sutherland, Ben <Ben.Sutherland@dfo-mpo.gc.ca>; Andrew Bateman <[REDACTED]>; Amy Teffer <[REDACTED]>; gideon mordecai <gmordecai@eoas.ubc.ca>  
**Cc:** Withler, Ruth <Ruth.Withler@dfo-mpo.gc.ca>; Beacham, Terry <Terry.Beacham@dfo-mpo.gc.ca>; Scott Hinch <scott.hinch@ubc.ca>; 'Suttle, Curtis' <suttle@science.ubc.ca>; Glenn Crossin <Glenn.Crossin@Dal.Ca>; [REDACTED] <[REDACTED]>; McLeod, Patricia <Patricia.McLeod@dfo-mpo.gc.ca>; MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>; Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>  
**Subject:** Away

[REDACTED]

I would appreciate being kept in the loop, and I do still check emails, but I would very much appreciate it if you guys can work together on decisions that need to be made and discussions on manuscripts, field sampling etc... I don't have a lot of bandwidth at present, and may not get things back that need to be read at the pace anyone would like. I can still take the odd call to deal with emerging issues.

Scott, you should probably remove me from any student committees/comprehensives/etc for the next month [REDACTED]

Ruth and Terry, one of you will need to act for me. Andrew, if you could take the lead with our epidemiology team, I would appreciate it. Karia and Amy, could you please lead the Quinsam sampling? Ben, sorry I did not get back in time on your proposal, [REDACTED] and after all, you are the lead! I am excited by the potential however. Terry, I am going to contact [REDACTED] and ask that you represent our lab for at least the GBC F2F. I know that he was concerned that they would have questions about the Fit Chips, so perhaps I can go over that with you in more detail, and

you can get more information on progress and potential on the MinION from Chris and Ben. [REDACTED] if you could connect with GBC and get an extension on our report due end of April, I would appreciate it.

I know there are other things I am supposed to be doing that I am not thinking of, so you can contact me if you have questions. My cell number is [REDACTED]

Thanks all,

Kristi

s.16(2)(c)

s.19(1)

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** April-11-19 10:17 AM  
**To:** Miller-Saunders, Kristi; Garver, Kyle  
**Cc:** [REDACTED]  
**Subject:** for you information - BCSRIF EOI from the ONA  
**Attachments:** BCSRIF- ONA High throughput pathogen monitoring.docx

Hello Kristi and Kyle,

The ONA has submitted the attached EOI to the BC Salmon Restoration and Innovation Fund (BCSRIF). The project is to implement the Fluidigm 46 pathogen assay in the ONA lab. We fully expect that this would change and develop as a project proposal with different partnerships but just wanted to ensure we have submitted an EOI that could be implemented off the shelf if needed.


**limləmt | Thank You**

[REDACTED]

**Okanagan Nation Alliance**  
101 – 3535 Old Okanagan Highway  
Westbank, BC V4T 3L7

**kt cpəlk stim' Hatchery**  
155 Enowkin Trail  
Penticton, BC, V2A 6J7

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**E** [REDACTED]

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## **1. Introduction**

### **1.1. Description of applicant**

The Okanagan Nation Alliance (ONA) was formed in 1981 as the inaugural First Nations government in the Okanagan, representing the 8 member communities including; Okanagan Indian Band, Upper Nicola Band, Westbank First Nation, Penticton Indian Band, Osoyoos Indian Band and Lower and Upper Similkameen Indian Bands and the Colville Confederated Tribes. ONA is a non-profit organization with a subsidiary (Okanagan Nation Aquatic Enterprises Ltd.) commercial enterprise.

ONA is a founding member of the Canadian Okanagan Basin Technical Working Group (COBTWG) and affiliated sub-committees, comprised primarily of ONA, Department Fisheries and Oceans Canada, and the Province of BC. ONA also belongs to (BOBTWG), composed of the aforementioned Canadian agencies plus U.S. counterparts (US Fish & Wildlife, Public Utilities Districts).

### **1.2. Collaboration and engagement**

Provide a brief background on the project and its significance to the priority themes of BCSRIF. Who was engaged in the development of this expression of interest?

Okanagan Nation Alliance has a highly successful history of restoring salmonid species in the Okanagan Basin through innovative hatchery practices, habitat enhancement, and fisheries management. ONA has used best available science to adaptively manage the aquatic community via ongoing monitoring and evaluation. In support of the salmon restoration activities the ONA has established a 2500 sq. ft. Aquatic Containment Level 2 compliant laboratory at the *kl̓c̓p̓alk̓ st̓im* Hatchery in Penticton, BC. The ONA laboratory has developed and implemented a disease testing facility to enable compliance with the terms of the aquaculture and transfer licenses for Sockeye and Chinook salmon enhancement activities. This facility provides the infrastructure for external and independent 3rd party monitoring of aquaculture activities that will be developed further through this project.

Infectious disease is considered to contribute to declines in wild salmon populations and to the success of salmon enhancement activities, but the distribution and prevalence of infectious agents causing diseases at various life stages of salmon is mostly unknown. This project presents the opportunity to address this knowledge gap through implementation of a DFO developed high-throughput microfluidics platform to screen for 45 infectious agents of concern for salmon. The tool will be applied to a range of activities including disease testing from contributing brood stock and 'in-lake' surveillance for emerging pathogens and pathogens of concern.

The principle project output includes increased capacity with ONA to provide internal and external disease monitoring. The disease monitoring activities will improve our knowledge and understanding of impacts to wild stocks through enhancement aquaculture activities. Direct outcomes will inform management decisions to improve sustainability of the enhancement aquaculture industry to ensure the protection, conservation and restoration of wild fish populations.

Who will be collaborating on the delivery of the project, if approved?

ONA collaborates with Federal, Provincial, and Municipal governments through various technical and steering committees. ONA is a founding member of the Canadian Okanagan Basin Technical Working Group (COBTWG) and affiliated sub-committees, comprised primarily of ONA, Department Fisheries and Oceans Canada, and the Province of BC. ONA also belongs to (BOBTWG), composed of the aforementioned Canadian agencies plus U.S. counterparts (US Fish & Wildlife, Public Utilities Districts). In addition, ONA is affiliated with the First Nation Fisheries Council and maintains a number of seats on the Aquaculture subcommittee including Tier 2 discussions with DFO Aquaculture. Direct collaborations with DFO Science Branch and DFO Pacific Region will be part of the project.

Will the project provide a benefit to the fish and seafood sector or contribute to the protection and restoration of wild BC priority fish stocks?

This project will provide the ONA laboratory with increased pathogen testing capability in support of Sockeye, Chinook and Sturgeon aquaculture activities, provide comparative data on the time course of pathogen prevalence between wild and enhanced salmon, and increase capacity within the ONA to provide external, independent 3rd party facility for monitoring of marine aquaculture activities. In addition, the ONA has developed a business case for the development of an indigenous centre of excellence for firsts nation communities across BC by enabling hosting and training of individuals from participating first nation communities to carry out laboratory services.

## **2. Description of initiative**

The project has been divided in to three Objectives to a phased delivery of work.

### **Objective 1 - Implementation of microfluidics tools**

Purchase, installation and training on Fluidigm microfluidics platform according to the following:

- Juno™ system, including instrument, MX Interface Plate, Barrier Tape Applicator and
- Juno™ HX Interface Plate for use with 96.96 Dynamic Array™ and Flex Six™ IFCs (integrated fluidic circuits).
- BioMark HD Reader Includes: 20 seats Analysis Software: Real-Time PCR, Digital PCR, Genotyping

Implement the 46 pathogen assay as developed and validated in Miller, K.M., Gardner, I.A., Vanderstichel, R., Burnley, T., Schulze, A.D., Li, S., Tabata, A., Kaukinen, K.H., Ming, T.J., Ginther, N.G. 2016. Report on the Performance Evaluation of the Fluidigm BioMark Platform for High Throughput Microbe Monitoring in Salmon. DFO Can. Sci. Advis. Sec. Res. Doc. 2016/038. xi +282 p.

#### **Objective 2 - Application to aquaculture monitoring**

The ONA laboratory completes testing for diseases of concern from the annual brood stock collection. Duplicate samples will be collected from the October 2019 brood stock to provide comparative data on the prevalence of Infectious hematopoietic necrosis virus (IHNV), Piscine orthoreovirus (PRV) and *Renibacterium salmoninarum* (the causative agent of Bacterial kidney disease (BKD)). This assessment will demonstrate the utility of the tool in the ONA laboratory staff.

#### **Objective 3 - Comparative Surveillance Study**

The Okanagan has experienced dramatic variations in climatic conditions over the past years from record drought to record floods. Throughout this time frame, ONA has archived tissue samples (brain and spleen/kidney) from juvenile Sockeye salmon collected at three times per year from both Skaha and Osoyoos Lakes. This cohort of tissue samples will undergo total nucleic acid extractions followed by testing for all 46 pathogens on the microfluidics platform. These data will enable pathogen prevalence estimates to compare the population fish health of the enhanced stock from Skaha Lake with the wild stock from Osoyoos Lake. These samples represent a unique opportunistic cohort for assessment of pathogen prevalence through the life cycle of juvenile salmon under dramatically variable annual climatic conditions.

### **3. Timeline**

The project is turn key and ready to be implemented at the time of funding approval. Quotes have been obtained from equipment providers and facility plans are in place. Samples for the comparative prevalence study have been collected and archived in preparation for future funding. The hatchery health management plan includes disease testing of brood stock at collection annually in October.

The project is expected to take one year from implementation to completion with the following milestones:

- Milestone 1- Installation and validation of equipment - Project month 4
- Milestone 2 - Implementation of the assays within the ONA lab - Project month 8
- Milestone 3 - Screen October brood stock samples for 46 pathogens - Project month 9
- Milestone 4 – Complete testing of juvenile in-lake samples - Project month 12

### **4. Objective(s)**



*BC Salmon Restoration Initiative Fund – Expression of Interest  
Okanagan Nation Alliance, 2019*

ONA's proposed Expression of Interest will support the increased productivity and sustainability of Sockeye and Chinook salmon and be available to other initiatives including the Upper Columbia White Sturgeon Recovery. The project will enable a greater understanding of the impacts of disease on these critical populations and support the protection and restoration of SARA listed endangered and threatened stocks. In addition, the outcomes of the juvenile screen will provide information on the impacts of disease on the interaction of wild and enhanced salmon populations.

### **Expected outcomes**

ONA's proposed project will provide critical infrastructure to enable provision of high throughput pathogen detection within a first nation laboratory for monitoring of aquaculture activities. The implementation of this tool in the ONA laboratory provides significant testing capacity to be available as an external independent laboratory for pathogen screening. Application of the tool to the sample set available provides demonstration of efficacy as well as informative research outputs that would be beneficial to enhancement activities throughout BC.

## **5. Financial Information**

**Table 1: Project budget**

<b>Project expenses (summary)</b>	<b>Cost estimates (\$)</b>
Capitol Equipment	400,000
Consumables	121,000
Labour	116,500
Total project costs	637,500

**Table 2: Project funding sources**

<b>Project funding summary</b>	<b>Amount of funding</b>	<b>Status (applied/confirmed)</b>
Applicant(s) contribution	95,625	Confirmed
Support from industry		

Support from other sources (specify)

Support requested from BCSRIF	541,875	Applied
Total project funding	637,500	

## **6. Deliverables**

What are the project deliverables?

1. Implementation of a high-throughput microfluidics platform to screen for 45 infectious agents of concern for Pacific salmon.
2. Report on comparative surveillance study on wild and enhanced juvenile Sockeye salmon from Skaha and Osoyoos Lakes.

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** April-26-19 9:56 AM  
**To:** Withler, Ruth  
**Cc:** MacDougall, Lesley  
**Subject:** RE: data and summary request - Chinook and PRV

I will start dealing with this request. Unfortunately Emiliano is away, so I hope I have what I need from his challenge study. I will cc both of you.

Kristi

---

**From:** Withler, Ruth  
**Sent:** April 26, 2019 8:06 AM  
**To:** Miller-Saunders, Kristi  
**Cc:** MacDougall, Lesley  
**Subject:** FW: data and summary request - Chinook and PRV

You can deal with Jay directly if you wish, but keep me and Lesley copied on things. If you want me to respond, let me know,  
R

**From:** Kreiberg, Henrik <Henrik.Kreiberg@dfo-mpo.gc.ca>  
**Sent:** April-26-19 7:39 AM  
**To:** Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>; MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>; Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Withler, Ruth <Ruth.Withler@dfo-mpo.gc.ca>  
**Cc:** Burgetz, Ingrid <Ingrid.Burgetz@dfo-mpo.gc.ca>  
**Subject:** RE: data and summary request - Chinook and PRV

Hi Jay – we'll do our best to cover in Kristi's ongoing absence. As of Monday, Ruth Withler is acting for Lesley, who returns May 6; it may be that the response comes from Ruth.  
Best/HK

*Henrik Kreiberg*

Head, Applied Technologies Section, Aquatic Diagnostics, Genomics & Technologies Division  
Dept. of Fisheries & Oceans, Government of Canada  
Pacific Biological Station, Nanaimo BC Canada V9T 6N7  
Tel 250-756-7019 (fax 7053) [henrik.kreiberg@dfo-mpo.gc.ca](mailto:henrik.kreiberg@dfo-mpo.gc.ca)

Chef de section, Technologies appliquées,  
Division des diagnostics aquatiques, génomique et technologies,  
Pêches et Océans Canada / Gouvernement du Canada  
Station biologique du Pacifique, Nanaimo CB Canada V9T 6N7  
Tel 250-756-7019 [henrik.kreiberg@dfo-mpo.gc.ca](mailto:henrik.kreiberg@dfo-mpo.gc.ca)

**From:** Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>  
**Sent:** 2019–April-25 6:10 PM

**To:** MacDougall, Lesley <[Lesley.MacDougall@dfo-mpo.gc.ca](mailto:Lesley.MacDougall@dfo-mpo.gc.ca)>; Higgins, Mark <[Mark.Higgins@dfo-mpo.gc.ca](mailto:Mark.Higgins@dfo-mpo.gc.ca)>; Kreiberg, Henrik <[Henrik.Kreiberg@dfo-mpo.gc.ca](mailto:Henrik.Kreiberg@dfo-mpo.gc.ca)>  
**Cc:** Burgetz, Ingrid <[Ingrid.Burgetz@dfo-mpo.gc.ca](mailto:Ingrid.Burgetz@dfo-mpo.gc.ca)>  
**Subject:** data and summary request - Chinook and PRV  
**Importance:** High

Hi Henrik,

In support of the Department's response to the recent court case, one of the analysis pieces we are collectively working on is to review all of the available data related to PRV and specifically Chinook salmon.

Given that Kristi's SSHI project has been conducting a challenge study with Chinook that has been recently completed, and she has intimated that there are other relevant data that has not yet been published, it is critical that these results are included in the summary that is being put together to support the Department's analysis.

I understand that Kristi may not be available directly, so as you are acting for Lesley, and Lesley has been the Science-Pacific lead on the response, I'm asking you if you can obtain a summary of the data and results from Kristi's lab on PRV and Chinook. The challenge study results are of particular relevance, as are the other data. So that there can be a critical and comprehensive up-to-date review, and given that the challenge study has not yet been published, the underlying data will also be critical.

We will be looking to convene some calls involving DFO scientists, including Kristi, to review the state of knowledge and provide a comprehensive summary to the decision-makers about what is known regarding PRV and Chinook.

As you can appreciate, there is very little remaining time prior to the decision-maker needing to make an informed decision, so any immediate support you can provide would be appreciated.

Thanks for your help,

Jay

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** April-26-19 12:09 PM  
**To:** Schulze, Angela  
**Subject:** RE: EIBs

I seemed to get the same email twice. Are there more files? Still not seeing the one I am looking for, but it may be the one with 7z on the end that I cannot open. Can you check this?

Kristi

**Sent:** April 23, 2019 10:18 AM  
**To:** Miller-Saunders, Kristi  
**Cc:** Schulze, Angela  
**Subject:** EIBs

Kristi,

Here is the folder that was under **D:\Kristi\PRV-related Diseases--EIBS- and Atlantic salmon transplant history**. I will also include some other files in other folders named with EIBs (**D:\Kristi\Kristi's Grants\GBC SSHI Pathogen Initiative\Histopathology -see also Histology on pub\EIBS Research** and **D:\Kristi\Kristi's Grants\GBC SSHI Pathogen Initiative\Histopathology -see also Histology on pub\In Situ Manuscript\EIBS Linkages**). Let me know if you receive this or if I need to forward it to some other address.

Angela

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

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## Miller-Saunders, Kristi

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**From:** Withler, Ruth  
**Sent:** April-26-19 12:13 PM  
**To:** Kreiberg, Henrik; Miller-Saunders, Kristi  
**Subject:** RE: Follow up from PRV call today

Henrik,  
Likely has something to do with folks in Mark's group,  
R

**From:** Kreiberg, Henrik <Henrik.Kreiberg@dfo-mpo.gc.ca>  
**Sent:** April-26-19 12:10 PM  
**To:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Withler, Ruth <Ruth.Withler@dfo-mpo.gc.ca>  
**Subject:** RE: Follow up from PRV call today

Thanks both, will figure something out.  
HK

**From:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Sent:** 2019–April-26 12:06 PM  
**To:** Withler, Ruth <Ruth.Withler@dfo-mpo.gc.ca>; Kreiberg, Henrik <Henrik.Kreiberg@dfo-mpo.gc.ca>  
**Subject:** RE: Follow up from PRV call today

No idea what the disease agent support tool is or what they are doing on aggregates. Nobody has our data as far as I know and that will take some putting together.

Kristi

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**From:** Withler, Ruth  
**Sent:** April 26, 2019 11:54 AM  
**To:** Kreiberg, Henrik  
**Cc:** Miller-Saunders, Kristi  
**Subject:** RE: Follow up from PRV call today

Don't think there is any overlap. Kristi: does the "Disease Agent Support Tool" refer to anything of yours?  
Ruth

**From:** Kreiberg, Henrik <Henrik.Kreiberg@dfo-mpo.gc.ca>  
**Sent:** April-26-19 11:32 AM  
**To:** Withler, Ruth <Ruth.Withler@dfo-mpo.gc.ca>  
**Subject:** FW: Follow up from PRV call today

Hi Ruth – not sure how much this query below overlaps with Jay's interest in Kristi's findings; one-liner on that?  
Mark suggests that John/StAR will lead on the aggregates point, but I have no sense re "Disease Agent Support Tool". Is that term known to you?  
HK

**From:** Kreiberg, Henrik  
**Sent:** 2019–April-26 7:42 AM

**To:** McCorquodale, Brenda <[Brenda.McCorquodale@dfo-mpo.gc.ca](mailto:Brenda.McCorquodale@dfo-mpo.gc.ca)>; Parsons, Jay <[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca)>  
**Cc:** Lowe, Carmel <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>  
**Subject:** RE: Follow up from PRV call today

Hi Brenda – referred this to Mark Higgins who is providing Sci. Br. continuity on the PRV file; will follow up with him this morning.  
Thanks/HK

## *Henrik Kreiberg*

Head, Applied Technologies Section, Aquatic Diagnostics, Genomics & Technologies Division  
Dept. of Fisheries & Oceans, Government of Canada  
Pacific Biological Station, Nanaimo BC Canada V9T 6N7  
Tel 250-756-7019 (fax 7053) [henrik.kreiberg@dfo-mpo.gc.ca](mailto:henrik.kreiberg@dfo-mpo.gc.ca)

Chef de section, Technologies appliquées,  
Division des diagnostics aquatiques, génomique et technologies,  
Pêches et Océans Canada / Gouvernement du Canada  
Station biologique du Pacifique, Nanaimo CB Canada V9T 6N7  
Tel 250-756-7019 [henrik.kreiberg@dfo-mpo.gc.ca](mailto:henrik.kreiberg@dfo-mpo.gc.ca)

**From:** McCorquodale, Brenda <[Brenda.McCorquodale@dfo-mpo.gc.ca](mailto:Brenda.McCorquodale@dfo-mpo.gc.ca)>  
**Sent:** 2019–April-25 4:09 PM  
**To:** Kreiberg, Henrik <[Henrik.Kreiberg@dfo-mpo.gc.ca](mailto:Henrik.Kreiberg@dfo-mpo.gc.ca)>; Parsons, Jay <[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca)>  
**Cc:** Lowe, Carmel <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>  
**Subject:** Follow up from PRV call today

Hi Henrik and Jay  
Just a note to follow up on the PRV call earlier today that Wayne Moore was on. I was tasked with contacting you to see where the paper on aggregates of salmonid stocks is (for incorporation with the practitioners' guide) and what the eta is for a draft of the Disease Agent Support Tool.  
Thanks  
Brenda

Brenda McCorquodale

A/ Director, Aquaculture Management (April 18 – May 15, 2019)  
Regional Manager, Aquaculture Resource Management  
Fisheries and Oceans Canada  
Gestionnaire régionale des ressources, Direction des pêches  
Pêches et Océans Canada

1965 Island Diesel Way | Nanaimo, BC | Nanaimo, CB | V9S 5W8  
Email | Courriel: [Brenda.McCorquodale@dfo-mpo.gc.ca](mailto:Brenda.McCorquodale@dfo-mpo.gc.ca)  
Telephone | Téléphone: 250-754-0367

## Miller-Saunders, Kristi

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**From:** Schulze, Angela  
**Sent:** April-26-19 1:21 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** FW: EIBS disease

Here is the email that was in the folder

Angela

**From:** Meyers, Theodore R (DFG) <ted.meyers@alaska.gov>  
**Sent:** October 16, 2018 8:03 AM  
**To:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Subject:** RE: EIBS disease

Hello Kristi,

The EIBS we reported on was the only case in our files for 30+ years. We maintain a close dialogue with all the hatcheries and routinely sample fish, so EIBS is quite rare in Alaska. This is despite that we have molecular evidence of PRV in several Alaskan salmonid stocks.

Unfortunately, we do not have any archived tissues from that one case.

Best of luck in your EIBS research, it will be interesting to see what you find.

Sincerely,

Ted Meyers, Ph.D.  
Principal Fish Pathologist  
ADF&G

**From:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Sent:** Sunday, October 14, 2018 2:05 PM  
**To:** Meyers, Theodore R (DFG) <ted.meyers@alaska.gov>  
**Subject:** EIBS disease

Hello Dr. Meyers,  
My colleagues (Hugh Ferguson and Emiliano Di Cicco) and I are carrying out a study that is attempting to trace the history of PRV detection in western North America and its potential linkage with EIBS disease. As you likely know, the Japanese found a cause and effect relationship between PRV-2 and EIBS, and they feel strongly that the disease they observe is the same as that first described in Washington State. In perusing the literature, I came across your 2007 study documenting the first case of EIBS in Alaska, and I was interested in whether EIBS continued to persist after that initial finding, and whether anyone has conducted further work into the etiology of the disease in Alaska.

We recently published a paper that utilized in situ hybridization to study the localization of PRV along the developmental pathway of HSMI in farmed Atlantic salmon and jaundice/anemia in farmed Chinook salmon (EIBS also present), and showed that the virus was intimately associated with the regions where lesions developed for both diseases (Di Cicco et al. 2018). We are interested in exploring a similar approach with EIBS occurring around the world, and are looking for collaborators who may have access to preserved tissues/blood for molecular analysis and histology blocks or blood smears for in situ analysis/histo staining.



Is this something that could interest you? If there are no preserved samples available, we would be happy to aid in the collection of samples if any outbreaks do occur over this next year. While EIBS has not been specifically described in BC hatcheries, we are looking into the potential that it is there but unrecognized in a few hatcheries with high levels of PRV.

Thanks for considering this request,

Kristi Miller  
Head, Molecular Genetics  
Pacific Biological Station  
Fisheries and Oceans Canada  
[Kristi.saunders@dfo-mpo.gc.ca](mailto:Kristi.saunders@dfo-mpo.gc.ca)

No information has been removed or severed from this page

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** April-29-19 1:02 PM  
**To:** Withler, Ruth  
**Subject:** RE: prv stuff

I have not sent anything as emiliano has been away and I don't have the data. I will deal with it later this week. Not sure how much i will have to provide as we have not analysed the data yet and i believe we have not even completed collecting it from the challenge.

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**From:** Withler, Ruth  
**Sent:** April 29, 2019 11:32 AM  
**To:** Miller-Saunders, Kristi  
**Subject:** prv stuff

I'll be on a PRV/framework call tomorrow – did you send any material to Jay or anyone else that I should be aware of? If you didn't, do you want me to give a time when you could participate (later this week or next week)?, R

## Miller-Saunders, Kristi

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**From:** Parsons, Jay  
**Sent:** May-03-19 12:35 PM  
**To:** Miller-Saunders, Kristi; Waddington, Zac  
**Cc:** Craig Stephen; Olivier, Gilles; Mimeault, Caroline; Burgetz, Ingrid; Weber, Lily  
**Subject:** PRV characterization paper for your official review  
**Attachments:** PRV Characterization Paper - Table of Changes.pdf; PRV Characterization paper 2019-05-03.pdf

### On the behalf of the PRV CSAS Co-chairs (Craig and Gilles)

Dear Kristi and Zac,

We are contacting you about the DFO CSAS peer-review process for the PRV risk characterization paper that took place in January 2019. As the official reviewers identified at the CSAS meeting please review to determine if the requested changes from the meeting have been incorporated appropriately. Please send your review by **Thursday May 9<sup>th</sup>, 2019**. Your roles as official reviewers are vital and greatly appreciated.

See the attached revised version of the PRV characterization paper including the incorporated changes requested at the CSAS peer-review meeting. A table of requested changes and how they were addressed has also been included for your reference.

Please recall we are not seeking a full re-review of the paper but rather a verification that the requested changes were incorporated and the responses are appropriate for the requested change.

Thank you,

Jay

### Jay Parsons, PhD

#### Director

Aquaculture, Biotechnology and Aquatic Animal Health Sciences Branch  
Fisheries and Oceans Canada / Government of Canada  
200 Kent Street, Stn 12E239 Ottawa, ON Canada K1A 0E6  
Jay.Parsons@dfo-mpo.gc.ca/ Tel. 613-990-0278

#### Directeur

Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques  
Pêches et Océans Canada / Gouvernement du Canada  
200 Kent Street, Stn 12E239 Ottawa, ON Canada K1A 0E6  
Jay.Parsons@dfo-mpo.gc.ca / Tél. 613-990-0278



Government  
of Canada

Gouvernement  
du Canada

Canada

## Changes for Authors

**National CSAS Peer Review Meeting:** Assessment of the risk to Fraser River sockeye salmon due to Piscine Orthoreovirus (PRV) transfer from Atlantic salmon farms located in the Discovery Islands area, British Columbia  
**Date:** January 28-30, 2019  
**Research Document:** PRV Characterization Paper

No	Section / Type of Comment	Comment / Considerations	Action Item	Changes made
1.	Overall	Review text to ensure descriptions are clear of bias and clarify text where indicated by reviewer. Specifically Line 33-37, 52, 62, 215, 223, 240, and 748.	Refer to Espen's written review to identify specific text where language was vague are requires clarification	Wording has been amended as suggested by the reviewer on the line numbers indicated.
2.	Overall/throughout	PRV1 should be PRV-1	Make change throughout all documents.	PRV1 has been changed to PRV-1 throughout. Similarly, PRV2 to PRV-2 and PRV3 to PRV-3 have been applied for consistency.
3.	General	PRV is not globally distributed, as well displayed in Fig 1. It is not reported from Africa, Australia, large parts of Asia.	Revise text to reflect PRV has been found in multiple countries but is not globally distributed.	The term 'is globally distributed' has been changed to 'is distributed across multiple countries around the world' for specific accuracy. The word 'global' has also been removed from Fig 1 legend.
4.	Line 31	What is meant by "salmon"? Any species of genera Salmo and Oncorhynchus? Only those that are anadromous?	Revise text for great clarity regarding the use of "salmon"	'salmon' has been changed to 'farmed Atlantic and Pacific salmon' for specificity.
5.	Line 33	What is meant by notable disease?	Revise text for clarity	'notable' has been removed
6.	Line 35	Causation studies now exist for each PRV subtype (1,2, and 3)	Update text and references	'at least two' has been amended to 'all three known genetic' and

No	Section / Type of Comment	Comment / Considerations	Action item	Changes made
				reference to Vendramin et al 2019 has been included.
7.	Line 38	Revise "reovirus infections..." to "piscine orthoreovirus infections..."	Revise accordingly	The use of reovirus rather than piscine orthoreovirus was intentional in this instance; it is meant to encompass data and studies on both ortho and aquareoviruses. 'both aquareovirus and orthoreovirus' has been added to clarify this.
8.	Line 81	PRV genus. PRV is a species belonging to orthoreovirus genus	Revise accordingly	'PRV genus' has been amended to 'PRV species' as indicated.
9.	Line 123	Add citation (Olsen, Hjortaas, Tengs, Hellberg & Johansen 2015; Hauge, Vendramin, Taksdal, Olsen, Wessel, Mikkelsen, Alencar, Olesen & Dahle 2017)	Revise accordingly	References have been added as suggested.
10.	Fig 1	Update figure to reflect detections of PRV-3 in Germany and France, PRV-1 in Denmark and Sweden	Update figure to current findings	Figures 1 as well as Table 1 have been updated to include PRV detections in France, Germany, Denmark and Sweden along with appropriate references as indicated.
11.	PRV-1 Biology Line 109-11	A description of the genetic diversity of PRV-1 in BC was included however the authors should also mention the potential for segment reassortment as a mechanism available to the virus for genome change	Add info accordingly	The sentence 'It is currently unknown if or how much segment reassortment occurs between genomic PRV variants in British Columbia.' has been added to the end of the paragraph.

No	Section / Type of Comment	Comment / Considerations	Action item	Changes made
12.	Line 119-120	<p>PRV2 is currently not known to occur in British Columbia or in the Eastern Pacific at large". That statement may only be partially true. EIBS has a long history of occurrence in Coho and Chinook salmon in the Pacific Northwest including other geographic areas, most notably Atlantic salmon in Norway and Coho Salmon in Japan as indicated earlier in this section. There is a strong likelihood that EIBS in the Pacific Northwest may be caused by a yet to be determined strain of PRV.</p> <p>PRV subtypes rely on specific qPCR assays for detection. Have assays in addition to PRV-1 been used to test EIBS samples from the Pacific Northwest?</p>	<p>The authors should discuss this and provide information (if available) to better understand the link of PRV and EIBS in the PNW.</p>	<p>The following text has been added to discuss the diagnosis of EIBS and the potential role of PRV-2 in this condition in North America:</p> <p>'Although an erythrocytic inclusion body syndrome associated with anemia (also abbreviated EIBS) has long been diagnosed in Coho and Chinook Salmon of the North Eastern Pacific (Arakawa et al. 1989), PRV-2 RNA could not be detected in at least one population of Coho Salmon manifesting this North American version of EIBS in Washington State (M. Purcell, personal communication) indicating PRV-2 is unlikely to be the primary causative agent responsible for this syndrome in North America.'</p>
13.	<p>Headings: Line 138</p> <p>Line 163</p> <p>Line 205-209</p>	<p>The previous section heading is "Host Range of PRV1". For clarification and consistency, the next section heading on Line 138 should include the wording of PRV1 as well, ie "Cellular Tropism of PRV1"</p> <p>same comment- "Infection Dynamics of PRV1"</p>	<p>When appropriate, revise with the use of PRV-1 to clarify that text is specific to PRV-1 or indicate that discussion is general to all PRV types.</p>	<p>Titles have been amended as suggested.</p>

No	Section / Type of Comment	Comment / Considerations	Action Item	Changes made
		<p>heading should be" <u>General</u> Pathogenicity of PRV" since the paragraph appears to discuss PRV in general followed by other sections specifically discussing each of the three genotypes. If this is true, then mention of PRV in Line 209 should be preceded by "Pacific Canadian" or some other qualifier to indicate the specific PRV strain.</p>		
14.	Line 149-152	<p>The authors suggest uncertainty regarding evidence for PRV replication in various cells. Detection of single stranded RNA through in situ hybridization may provide insight into PRV tissue tropism and should be discussed.</p>	Add info accordingly	<p>The published in situ hybridization method used temperatures &gt;90° for &gt;5 min prior to probe hybridization on PRV RNA (Di Cicco et al 2018). Temperatures ≥ 90°C for ≥5 min have been shown to denature PRV genomic dsRNA into ssRNA components (Polinski et al. 2019). Therefore, as originally stated, it remains unclear as to whether the hybridization assay is detecting replicating virus (i.e., mRNA rather than denatured gRNA). Further, generation of mRNA is not definitive evidence that complete replication (generation of infectious particles) has been accomplished and all attempts to increase viral load in the cell types referenced in vitro have failed (Ln167-172 track</p>

No	Section / Type of Comment	Comment / Considerations	Action item	Changes made
				changes document) further supporting the unclear ability for PRV to replicate in these cells. This detailed explanation is anticipated to confuse rather enhance general readership comprehension; we therefore have refrained from adding it to the final document.
15.	Line 178	When referring to the term “dose”, it should be explained that RNA levels measured by qPCR do not differentiate between full virions, ISVP (infective subviral particles) and core particles of which only two are infectious.	Add explanation	‘PRV isolations at a similar dose’ has been changed to ‘PRV isolations with similar RNA loads’ for specific accuracy.
16.	Line 199	Along with the discussion regarding the timing of the occurrence of inflammation in relation to the PRV infection cycle, the authors should discuss that the amount of virus and virus Ct values are likely correlative with the amount of virus protein until the peak of infection, and thereafter not corresponding.	Add text accordingly	The current methods for estimating viral protein quantities do not allow for direct correlative analysis with genomic material because it is unknown if or at what efficiency the monoclonal antibodies designed to detect individual recombinant PRV proteins detect those proteins once they are assembled into an infectious PRV particle.
17.	Line 240 and throughout	the term “isolation” suggests cell culture which is not correct- rather it should be changed to “purified isolate”.	Revise accordingly to indicated what isolate is defining in this instance where virus was not cultivated.	‘isolation’ has been changed to ‘purification-based isolation’ for accuracy.



No	Section / Type of Comment	Comment / Considerations	Action item	Changes made
18.	Line 242	similar clarification "... PRV1a and PRV1b have been <u>physically</u> isolated from..."	Revise accordingly	'isolated' has been changed to 'physically isolated' as suggested.
19.	Lines 254	"...indicating that factors specific to the commercial field environment in Norway contribute to HSMI"  Please refer to Kristoffersen et al 2013 as evidence for environmental influence of HSMI prevalence in Norway. Explain that geographic areas within Norway have varying levels of HSMI	Provide appropriate literature or adjust wording. Suggestion from reviewer: HSMI increases in prevalence with increasing cohort lifespan, increasing infection pressure and increasing cohort size, and local geography (Kristoffersen et al 2013).	The information suggested by the reviewer has been incorporated as follows:  '...with or without hypoxic stress (Lund et al. 2017, Wessel et al. 2017) – indicating that factors specific to the commercial field environment in Norway contribute to HSMI. This is supported by previous finding that HSMI increases in prevalence with increasing cohort lifespan, infection pressure, cohort size, and local geography (Kristoffersen et al 2013).'
20.	Line 255	Adjust text to indicate that the AquaGen fish had less heart damage and higher survival rather than indicating that they were "resistant to HSMI disease". Also remove reference to Mowi origin as it is unclear as to the current genetic composition of the AquaGen strain.	Adjust accordingly	'is resistant to HSMI disease' has been changed to 'had less heart damage and higher survival' as suggested; indication that this was a Mowi strain has been removed.
21.	Line 264	Qualify that the disease is rare in BC	Review and correct	'in BC' has been added for specificity.
22.	Line 276	Clarify that Takano et al reproduced anemia through	Adjust definition of disease accordingly	'and has caused moderate anemia in Coho Salmon following

No	Section / Type of Comment	Comment / Considerations	Action item	Changes made
		experimental challenge with purified PRV-2.		experimental infection' has been added for clarity.
23.	Fig. 2	Clarify that data presented represents <b>general trends</b> drawn from different experiments limiting direct comparisons due to multiple variables.		'trends' has been amended to 'general trends' as suggested. The additional wording 'Note that some variance in experimental design exists between these experiments.' has also been added for emphasis.
24.	Lines 379-381	The statement regarding vertical transmission is vague. Is it surface contamination by the virus that infects the alevin when it hatches? OR.. is the virus actually entering the egg through the micropyle (with sperm or during water hardening) to later infect the developing embryo (true vertical transmission)? A similar vague argument was made in the IHN V DFO risk assessment already published (Mimeault et al 2017) where surface egg associated virus can be mitigated in hatcheries by properly disinfecting the eggs. Such mitigation is not possible for true vertical transmission which cannot be circumvented by surface disinfection and/or periodic antifungal treatments.	Clarify in text	We have removed the ambiguous text about vertical transmission and have added the following text for clarity:  '...progeny from 2008 to 2011 found that PRV was not isolated in or on eggs collected from infected brood fish following a Buffodine® disinfection treatment (Wiik-Nielsen et al. 2012). Similarly, PRV could not be detected in juvenile Atlantic Salmon reared from iodine disinfected eggs at a commercial freshwater facility in British Columbia even though the brood fish supplying the facility carried high systemic RNA loads of PRV (Polinski & Garver, unpublished data). As iodine disinfects egg surfaces but not the internal yolk or embryo, this data suggests that infectious PRV is not carried internally within fertilized eggs of

No	Section / Type of Comment	Comment / Considerations	Action item	Changes made
				salmon and that, if PRV is present on egg surfaces, iodine-based disinfection has the potential to be used to block infectious PRV transmission from parent to offspring.'
25.	Line 381	Add " <u>Fish</u> in freshwater hatcheries...." since hatcheries (a non-living entity) do not become infected. Also, there should be a reference(s) provided to support infection by PRV in freshwater hatcheries in North America and Europe.	Clarify and reference	'Freshwater hatcheries' has been changed to 'Fish in freshwater hatcheries' and references have been provided as suggested.
26.	Line 387	Work by Teige et al 2017 Fish and shellfish immunology 63,491-499 may contribute information to shedding.	Review reference and add if appropriate	Teige et al 2017 demonstrated that antibodies against two PRV proteins were generated during infection using a bead-based multiplex immunoassay. As there was no indication in the paper as to whether antibody production affected PRV shedding (shedding was not measured in that study) there results do not directly add to discussions concerning shedding.
27.	Line 393	Indicate that is remains unknown as to what factors may impact virus shedding. For instance it is unclear as to whether a stress event may or may not influence virus shedding.	Adjust accordingly	'and it remains unknown as to what factors may impact virus shedding.' Has been added as suggested.

No	Section / Type of Comment	Comment / Considerations	Action item	Changes made
28.	Line 402-418	Stabilities for IPNV could be used as a proxy for PRV stability in the environment, however it needs to be noted that although IPNV is non enveloped it only contains 2 genomic segments while PRV contains 10 segments	Include text explaining potential use of IPNV stability as a proxy for PRV	'In contrast, infectious pancreatic necrosis virus (IPNV), a nonenveloped aquatic fish virus, requires days to lose infectivity in estuarine water (salinity 11.5 ppt) (Toranzo et al. 1983).' has been added as an example for non-enveloped viral stability; however, we have not suggested IPNV be used as a proxy for PRV given the differences in genomic content and capsid structure as well as due to the lack of any data to indicate whether PRV would have less, equal, or greater stability than IPNV.
29.	Line 499	Provide references for sentence.	Add references	A reference to table 1 has been added to indicate where all citations available to support this statement are provided. Clarity regarding the detection of PRV RNA rather than infectious particles has also been added for specific accuracy to support the findings of cited references.
30.	Line 606	Explain importance of defining the difference in disease causing potential between BC and Nor-PRV and why this useful to evaluating pathogenesis in other fish species such as Pacific salmon.	Include explanation where appropriate.	This section is not specific to PRV, but is a summary of cardiophathy (all causes) in salmon of BC. The reviewer's comment concerning the affects of different viral strains on different host species are discussed elsewhere [e.g., General Pathogenicity of PRV and Regional

No	Section / Type of Comment	Comment / Considerations	Action item	Changes made
				Variations in Virulence (PRV-1) sections].
31.	Line 693	How is anemia assessed in carcasses?	Provide explanation	'based on excessive internal organ pallor or jaundice' has been added to the sentence for clarity. Additionally, 'it should be noted that subclinical anemia would likely be missed by visual inspection of moribund or recently deceased specimens' to acknowledge that these diagnosis are not a guarantee of absolute prevalence.
32.	Line 747	Suggest adding the words (underlined) " .... contributing to jaundice/anemia in <u>captive fish</u> and may be ...."	Revise if appropriate	Wording has been amended as suggested.
33.	Line 748	Adding the sentence – <u>However, this assumption cannot be extrapolated to wild Chinook salmon or other Pacific salmon because captive and wild environments can cause different pathophysiological and susceptibilities.</u> This statement reinforces the statement already in Lines 801 and 802.	Revise if appropriate	Sentence has been added as suggested.
34.	Line 783	Note that the data were from experimental infections	Provide clarification	'experimental' has been added as suggested and 'juvenile' has also been added for further specific accuracy.
35.	Line 786	"The severity of a systemic PRV	Clarify text.	'The severity of a systemic PRV

No	Section / Type of Comment	Comment / Considerations	Action item	Changes made
		infection..."		infection is not...' has been changed to 'Systemic PRV load is not...' for clarity.
36.	Line 791	What is mean by severity here, is it load of virus?  Suggest adding the words to the existing sentence "... likely have complex etiologies and <u>have not been reported in wild Pacific salmon.</u> "	Revise if appropriate	Wording has been added as suggested.

Three additional modifications have been incorporated to the PRV Characterization Paper to address comments made regarding the Risk Assessment Paper and to reflect the findings of Fux et al 2019 which was distributed to all participants at the workshop:

- 1.) During the workshop, Jay Parsons circulated a study by Fux et al. (2019) which had just been published demonstrating that PRV-3 was not the causative agent of PDS in Brown Trout of Europe. We have therefore updated the Characterization paper (Ln318-324 track changes document) to reflect this new data which all participants were able to consider during the risk assessment proceedings.
- 2.) A reviewer comment to the Risk Assessment Paper was to indicate that true prevalence and detection prevalence are not necessarily homogenous and that various studies have used different sampling protocols, analyses and stringencies for determining pos/neg detection without comparative diagnostic validation which should be noted. We have thus amended 'prevalence' to either 'detection' or 'detection prevalence' where appropriate throughout this document for accuracy, and have included a statement to acknowledge that the various methods used for PRV screening may not be equally accurate for considering true prevalence (Ln545-548 track changes document).
- 3.) A reviewer comment to the Risk Assessment Paper questioned the stated assumption that 'Results from laboratory studies on the impact of PRV infection in Sockeye Salmon are indicative of what occurs in the marine environment'. We were requested to add a supportive statement to help define the rationale for this assumption in the Characterization Paper (Ln759-774 track changes document).



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**Characterization of piscine orthoreovirus (PRV) and associated diseases to  
inform pathogen transfer risk assessments in British Columbia**

Mark Polinski and Kyle Garver

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Nanaimo, British Columbia, V9T 6N7

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### **Foreword**

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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## ABSTRACT

Piscine orthoreovirus (PRV) is a common and pervasively distributed virus of salmon. In Canada, nearly all sea-farmed salmon likely become infected with PRV prior to harvest and the virus has been detected in archived specimens dating back to at least the mid 1980's in British Columbia. Wild salmon (all species) also occasionally become infected with PRV. Detection is generally lower in wild populations than on farms, and not all salmon species are equally susceptible to PRV infection. Specifically, Sockeye Salmon appear mildly refractory compared to other species such as Atlantic Salmon. Among the wild Pacific salmon species in the Eastern Pacific, Coho and Chinook Salmon have the highest prevalence of PRV (approximately 9% and 6%, respectively); this prevalence appears independent from whether fish were collected from locations in close proximity to salmon farming or from areas devoid of salmon farming. The cumulative prevalence of PRV detected in Sockeye Salmon of Western North America over the past decade is approximately 1.5% based on the sampling of nearly 7,000 specimens of which more than 6,000 were collected from British Columbia stocks. Nonetheless, laboratory studies demonstrate that PRV infected Atlantic Salmon (dependent upon stage of infection) can transmit virus to cohabitating Sockeye Salmon; although the minimum exposure time, dose, and whether such transmission requirements would be reached in natural environs remain unknown. In some farmed salmon, PRV has caused disease – namely, cardiopathy and/or anemia – particularly in Europe and Japan. In farmed salmon of British Columbia, on rare occurrences, PRV has been detected in diseased Atlantic and Chinook salmon where the virus may have contributed to or caused the disease. This includes at least one instance of severe cardiopathy in farmed Atlantic Salmon and one instance of anemia in farmed Chinook Salmon in the past decade. If or when disease may manifest as a result of PRV infection is not well understood, appearing to require complex etiological factors that include host, virus, and environmental components. Both regional as well as viral strain-specific variations in virulence have been documented, and disease has, as yet, only been identified in farmed salmon populations. Important to discussions of PRV in Canada is that PRV in the Eastern Pacific appears less virulent in comparison to PRV in the Eastern Atlantic, and experimental infection of Sockeye and Atlantic Salmon with the PRV strain endemic to the Eastern Pacific has failed to manifest significant disease or impact respiratory function even though extreme systemic blood infections developed in both species. Furthermore, stressors such as smoltification, hypoxia, exhaustive chasing, or secondary viral (infectious hematopoietic necrosis virus) superinfection of salmon have not induced or enhanced this PRV virulence. Thus, neither the presence nor quantity of PRV generated during an infection is indicative of disease or physiological impairment in salmon of British Columbia.

## INTRODUCTION

Fisheries and Oceans Canada (DFO) has a regulatory role to ensure the protection of the environment while creating the conditions for the development of an economically, socially and environmentally sustainable aquaculture sector. Restoring funding to support federal ocean science programs to protect the health of fish stocks, to monitor contaminants and pollution in the oceans, and to support responsible and sustainable aquaculture industries in Canada has been identified as a top priority of the Minister of Fisheries, Oceans and the Canadian Coast Guard.

It is recognized that there are interactions between aquaculture operations and the environment (Grant and Jones, 2010). One interaction is the risk to wild salmon populations resulting from the potential spread of infectious diseases from Atlantic Salmon (*Salmo salar*) farms in British Columbia (BC) (Cohen, 2012). While several Atlantic Salmon farms are located within the migratory routes of Pacific salmon species, no risk assessment has been conducted to specifically determine the risk to wild fish populations associated with pathogens released from Atlantic Salmon farms.

DFO Aquaculture Management Division requested formal science advice on the risks of pathogen transfer from Atlantic Salmon farms to wild fish populations in BC. Given the complexity of interactions between pathogens, hosts and the environment, DFO will deliver the science advice through a series of pathogen-specific risk assessments followed by a synthesis.

## PURPOSE OF THIS DOCUMENT

The information summarized in this document will assist in the environmental assessment of the risk to Fraser River Sockeye Salmon (*Oncorhynchus nerka*) due to the occurrence of piscine orthoreovirus (PRV) infection on Atlantic Salmon farms located in the Discovery Islands area of British Columbia. This document is designed to be a focused consideration on PRV as a potential causative or contributing agent of disease in salmon of British Columbia which might be presumed to occur and putatively impact Fraser River Sockeye Salmon. As a consequence, this document concentrates on data pertinent to the transmission, pathogenicity (potential for causing disease) and virulence (potential for disease severity) of PRV to Sockeye Salmon occurring in the Discovery Islands area.

## GENERAL CONSIDERATIONS

Reovirus infections of farmed Atlantic and Pacific salmon are widespread and nearly all farmed stocks likely become infected at some time during a production cycle. The vast majority of these infections do not result in disease. Nevertheless, in some instances disease syndromes of salmon have been associated with aquatic reovirus infections; specifically, field and laboratory studies with piscine orthoreovirus (PRV) have identified an etiological link between all three known genetic PRV types and circulatory diseases: cardiopathy (heart disease) and/or anemia (insufficient number of red blood cell or hemoglobin) (Takano et al., 2016; Wessel et al., 2017; Vendramin et al., 2019).

Reovirus (both aquareovirus and orthoreovirus) infections are also regionally ubiquitous in wild salmon, although prevalence in and across wild stocks are generally lower than among farms. To our knowledge, there is no direct evidence that reovirus infections (and specifically PRV infections) cause disease in populations of wild salmon. Nevertheless, indirect inference from the fact that reoviruses can sometimes cause disease in farmed salmon suggests that similar diseases may occur in wild salmon assuming all host, environmental, and pathogen specific factors can be fulfilled in a natural setting.

A chief consideration in assessing PRV related risks is that the potential for PRV to cause disease in farmed salmon appears to be a complex process with regional variability and high dependence on host, virus, and environmental factors (Garver et al., 2016a; Polinski et al., 2019). This complexity becomes further complicated by dynamic industry and natural field environments such as those found in the Discovery Islands Region of Canada. Recent scientific investigations have identified several putative factors involved in PRV-associated disease, but much is still unknown.

Importantly, PRV presents an atypical example of a microbial pathogen in that the quantity of virus generated during an infection is not an accurate predictor of whether a fish becomes diseased or how severe an associated disease becomes (Lund et al., 2017; Polinski et al., 2019; Zhang et al., 2019). This is counterpoint to most animal pathogens for which disease presence and severity is directly correlated with pathogen load. As a consequence, the risks associated with PRV on salmon health require careful and atypical considerations relative to other salmon pathogens currently of note in British Columbia.

In this document, we provide an overview of PRV and highlight its potential and variable ability to cause disease in salmon. We then review two disease states (cardiopathy and anemia) within farmed salmon of British Columbia for which there is indirect evidence that PRV might have the ability to be a contributing or causative factor. Finally, we discuss current knowledge about the potential interrelationship of PRV and these disease states in salmon of British Columbia and specifically how this relates to Sockeye Salmon. This review focuses considerably on one genogroup of PRV (PRV-1) because it is the only genogroup that has been detected in North America and is also the most well studied.

## PISCINE ORTHOREOVIRUS

### GEOGRAPHIC DISTRIBUTION AND GENETIC TYPES

PRV is a non-enveloped, double stranded RNA virus within the *Reoviridae* family (Palacios et al., 2010; Kibenge et al., 2013) that is distributed across multiple countries around the world (Figure 1). PRV has been generally accepted as a species within the orthoreovirus genus due to its 10 linear dsRNA segmented genome and phylogenetic ordination to other orthoreoviruses

(Markussen et al., 2013). However, distinction between the orthoreovirus and aquareovirus genus is currently not well defined and a need for taxonomic reassessment has been suggested given the common yet divergent ordination of PRV to both genera (Nibert and Duncan, 2013), the likely common ancestry of the two genera (Attoui et al., 2002), and the recent discovery of additional putative orthoreoviruses in multiple divergent fish lineages including cartilaginous fish (Shi et al., 2018). Of specific relevance to PRV is that this virus is phylogenetically distinct from other currently known species in both the aquareovirus and orthoreovirus genera with unique genotypic and phenotypic characteristics (Key et al., 2013; Roscow et al., 2018).

Within the current PRV species, more than 20 molecular isolations have yielded fully sequenced genomes. Phylogenetic analyses using amino acid and nucleotide sequences from multiple genomic segments suggest three distinct genogroups: PRV-1, PRV-2 and PRV-3 (Dhamotharan et al., 2018; Kuehn et al., 2018). Each genogroup appears to be loosely segregated by geographical and/or host species divisions, although exceptions exist, and to date isolates from multiple PRV genogroups have not been detected within a single individual host. Nevertheless, members of all three genogroups appear to specifically target salmon, have a proclivity for infecting red blood cells that lead to extensive systemic blood infections, and are suggested to be within a single genus based on current orthoreovirus taxonomic characterization (King et al., 2011; Markussen et al., 2013).

## PRV-1

PRV-1 was first identified in Norway (Palacios et al., 2010) and has since been ubiquitously detected in that country (Lovoll et al., 2012; Wiik-Nielsen et al., 2016). PRV-1 is also commonly detected in farmed Atlantic Salmon from Canada, Chile, the United Kingdom, Ireland, Iceland, Germany, Denmark, France and the United States with an additional single detection from an Atlantic salmon in Sweden (Table 1) (Biering and Garseth, 2012; Garseth et al., 2013; Kibenge et al., 2013; Marty et al., 2015; Siah et al., 2015; Garver et al., 2016b; Adamek et al., 2018; Labrut et al., 2018; Vendramin, 2019). Retrospective studies of archival specimens have identified a historical presence of PRV-1 in Atlantic Salmon in both Norway and Canada dating back to at least the mid 1980's, with presumed high prevalence in farmed populations during much of that time (Marty et al., 2015; Markussen et al., 2018). Phylogenetic comparisons of the PRV-1 S1 genomic segment – which codes the outer clamp protein  $\sigma 3$  of the viral capsid and displays high sequence heterogeneity between isolates – further suggests possible additional delineations within this genogroup. Specifically, PRV-1a and PRV-1b subgroups has been proposed (Kibenge et al., 2013). However, as more PRV-1 sequences become available, new preliminary evidence suggests that whole genome sequence comparisons may provide a clearer picture of PRV-1's divergent regional evolution than S1 alone (Siah et al., 2018), and may prove particularly significant as additional preliminary evidence suggests that segment reassortment may be occurring in Norway between subgroups; i.e., between PRV-1a and PRV-1b (Markussen et al., 2018). Of importance with regard to PRV in British Columbia is that there appears to be relatively high genome homology between PRV-1 isolates within the Eastern Pacific and that these isolates are notably distinct from isolates sequenced from PRV-1 in the Atlantic (Siah et al., 2015; Di Cicco et al., 2018; Siah et al., 2018; Polinski et al., 2019). It is currently unknown if or how much segment reassortment occurs between genomic PRV variants in British Columbia.

## PRV-2

The second PRV genotypic variant (PRV-2) is currently only associated with Coho Salmon (*Oncorhynchus kisutch*) in Japan (Takano et al., 2016), and to date has not been detected in

## PRV and Associated Disease

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any other country or fish species. Although the historic prevalence of PRV-2 is unknown, the presence of a disease condition associated with PRV-2 in Japan known as erythrocytic inclusion body syndrome or EIBS has been documented since at least the mid 1980's (Takahashi et al., 1992), suggesting PRV-2 has been present in Japan since that time. PRV-2 is currently not known to occur in British Columbia or in the Eastern Pacific at large. Although an erythrocytic inclusion body syndrome associated with anemia (also abbreviated EIBS) has long been diagnosed in Coho and Chinook Salmon of the North Eastern Pacific (Arakawa et al., 1989), PRV-2 RNA could not be detected in at least one population of Coho Salmon manifesting this North American version of EIBS in Washington State (M. Purcell, personal communication) indicating PRV-2 is unlikely to be the primary causative agent responsible for this syndrome in North America.

### PRV-3

The third PRV genotypic variant (PRV-3) was identified in farmed Rainbow Trout (*Onchorhynchus mykiss*) in Norway (Olsen et al., 2015; Hauge et al., 2017) and has subsequently been reported in farmed Coho Salmon in Chile and in farmed Rainbow Trout in several European countries including Denmark, Scotland, Germany, France, and Italy (Dhamotharan et al., 2018; Labrut et al., 2018). PRV-3 has also been detected in Brown Trout (*Salmo trutta*) from Germany (Kuehn et al., 2018). The historic presence of PRV-3 in these countries is unknown. PRV-3 is also not known to occur in British Columbia at this time.



Figure 1. Detection of PRV in natural and farmed fish populations by country and/or geographic region.

### HOST RANGE OF PRV-1

Natural infections and controlled laboratory exposure studies indicate PRV-1 predominately infects salmonid fish (Table 1). Occasional detection of PRV nucleic acid (RNA) has also been accomplished in some non-salmonid fish species of the North Atlantic and in Eulachon (*Thaleichthys pacificus*) in the Pacific, although none have shown indication of being a primary ecological host and their capacity to replicate or transmit PRV-1 remains unknown.

147 Table 1. Fish species in which PRV-1 genetic material has been detected.

Species	Scientific name	Reference
<b>Canada</b>		
Atlantic Salmon	<i>Salmo salar</i>	Kibenge et al. (2013)
Sockeye Salmon	<i>Oncorhynchus nerka</i>	Miller et al. (2014)
Chinook Salmon	<i>Oncorhynchus tshawytscha</i>	Garver et al. (2016b)
Coho Salmon	<i>Oncorhynchus kisutch</i>	Marty et al. (2015)
Pink Salmon	<i>Oncorhynchus gorbuscha</i>	Marty et al. (2015)
Chum Salmon	<i>Oncorhynchus keta</i>	Kibenge et al. (2013)
Steelhead Trout	<i>Oncorhynchus mykiss</i>	Kibenge et al. (2013)
Cutthroat Trout	<i>Oncorhynchus clarkii</i>	Kibenge et al. (2013)
Dolly Varden Trout	<i>Salvelinus malma</i>	Morton et al. (2017)
Eulachon	<i>Thaleichthys pacificus</i>	Hrushowy (2018)
<b>United States</b>		
Atlantic Salmon	<i>Salmo salar</i>	Warheit (2018)
Chinook Salmon	<i>Oncorhynchus tshawytscha</i>	Purcell et al. (2018)
Coho Salmon	<i>Oncorhynchus kisutch</i>	Marty et al. (2015)
Pink Salmon	<i>Oncorhynchus gorbuscha</i>	Marty et al. (2015)
Steelhead Trout	<i>Oncorhynchus mykiss</i>	Purcell et al. (2018)
<b>Norway</b>		
Atlantic Salmon	<i>Salmo salar</i>	Palacios et al. (2010)
Sea Trout	<i>Salmo trutta</i>	Garseth et al. (2013)
Great Silver Smelt	<i>Argentina silus</i>	Wiik-Nielsen et al. (2012)
Atlantic Horse Mackerel	<i>Trachurus trachurus</i>	Wiik-Nielsen et al. (2012)
Atlantic Herring	<i>Clupea harengus</i>	Wiik-Nielsen et al. (2012)
Capelin	<i>Mallotus villosus</i>	Wiik-Nielsen et al. (2012)
<b>Chile</b>		
Atlantic Salmon	<i>Salmo salar</i>	Kibenge et al. (2013)
Coho Salmon	<i>Oncorhynchus kisutch</i>	Godoy et al. (2016)
<b>Iceland</b>		
Atlantic Salmon	<i>Salmo salar</i>	Gunnarsdóttir et al. (2018)
<b>Ireland</b>		
Atlantic Salmon	<i>Salmo salar</i>	Rodger et al. (2014)
<b>Faroe Islands</b>		
Atlantic Salmon	<i>Salmo salar</i>	Markussen et al. (2018)
<b>Germany</b>		
Atlantic Salmon	<i>Salmo salar</i>	Adamek et al. (2018)
<b>France</b>		



Atlantic Salmon	<i>Salmo salar</i>	Labrut et al. (2018)
<b>Denmark</b>		
Atlantic Salmon	<i>Salmo salar</i>	Vendramin (2019)
<b>Sweden</b>		
Atlantic Salmon	<i>Salmo salar</i>	Vendramin (2019)

## 148 CELLULAR TROPISM OF PRV-1

149 The primary cell type targeted by PRV in salmon is the erythrocyte (red blood cell). Unlike  
 150 mammals, fish erythrocytes remain nucleated throughout their lifespan and thus possess the  
 151 cellular components necessary for viral replication during all cellular life stages. PRV is detected  
 152 with the highest prevalence in blood during most stages of infection relative to all other tissue  
 153 types tested (Finstad et al., 2014; Garver et al., 2016a), and of the three types of blood cells  
 154 (red blood cells, white blood cells and platelets), red blood cells appear to be the only cell type  
 155 significantly infected (Wessel et al., 2015; Polinski et al., 2019). Amplification of both PRV-1  
 156 protein and genetic material occurs within erythrocytes (Finstad et al., 2014; Wessel et al.,  
 157 2015) and erythrocytes have repeatedly been used to initiate experimental infections (Wessel et  
 158 al., 2015; Polinski et al., 2019). This provides strong empirical evidence that infectious virus can  
 159 be generated within this cell type. Secondary infections of cardiomyocytes (heart muscle cells),  
 160 enterocytes (intestinal absorptive cells) and tissue-resident leukocyte-like cells (presumably  
 161 macrophages) have also been reported (Di Cicco et al., 2017; Di Cicco et al., 2018). However, it  
 162 is unclear as to whether or not PRV replication occurs within these cell types, and *in vitro*  
 163 experimental infection of Atlantic Salmon heart endothelial (ASHe), epithelial (ASK) and  
 164 fibroblast (BAASf) laboratory cells lines, as well as Rainbow Trout macrophage (RTS11) and  
 165 approximately 20 other fish laboratory cells lines, have yet to effectively replicate PRV-1 under  
 166 varied environmental conditions (Pham, Bols, Polinski and Garver, unpublished data). One  
 167 laboratory cell line, GF-1, derived from the fin of orange-spotter grouper, *Epinephelus coioides*,  
 168 showed cytopathic effects suggestive of viral replication after being inoculated with a  
 169 homogenate containing PRV (Mikalsen et al., 2012). However, PRV was not visualized by  
 170 electron microscopy (Mikalsen et al., 2012) and subsequent attempts to detect amplification of  
 171 PRV in GF-1 cells using RT-qPCR proved negative (Garver et al., 2016b).

## 172 INFECTION DYNAMICS OF PRV-1

173 The kinetics of PRV-1 as observed in Atlantic Salmon indicates three distinct phases of  
 174 infection: early entry and dissemination, peak systemic replication, and long-term persistence. In  
 175 the first (early) phase of infection which typically lasts 2-3 weeks at 12°C, initial host entry,  
 176 replication and dissemination of the virus into blood cells occurs. It is unknown where PRV first  
 177 enters host cells, although it is likely through cells of the respiratory (gill) or enteric  
 178 (gastrointestinal) epithelium as these sites are typical for reovirus entry. Mammalian  
 179 orthoreoviruses first infect epithelial cells of the small intestine or lung prior to haematogenous  
 180 dissemination (Boehme et al., 2013); and the recent detection of PRV in intestinal enterocytes  
 181 (Di Cicco et al., 2018) indicates that a similar course of infection might be followed by PRV.  
 182 Upon infection, the early replicative phase of mammalian reovirus likely dictates how much virus  
 183 gets disseminated, ultimately setting the course and overall severity of infection (Lai et al.,  
 184 2013). This first phase appears equally important with PRV infections and may account for  
 185 discrepancies in total virus production occasionally observed following laboratory challenge of  
 186 salmon with different PRV isolations with similar RNA loads, where a lag in replication of one  
 187 isolate appears to be the major difference between otherwise identical replication dynamics with

blood cells (Polinski et al., 2019). A lack of PRV transmission via fish cohabitation at this early stage of infection also suggests that whatever cell type(s) PRV is initially infecting, it is not likely being shed into the environment to a high degree (Polinski et al., 2019).

In the second (peak) phase of infection that typically lasts 2-3 weeks at 12°C, substantial PRV replication within erythrocytes occurs along with the formation of cytoplasmic viral inclusions (Finstad et al., 2014; Wessel et al., 2015; Haatveit et al., 2017; Polinski et al., 2019) similar to those that develop during mammalian reovirus infection of well-established cell lines (Eichwald et al., 2018). The highest systemic blood loads of PRV occur during this period, and it is when innate virus recognition pathways of the host are most likely to become activated, although this activation can be variable and even nonexistent depending on PRV regional variants [summarized by (Polinski et al., 2019)]. Cohabitation challenges have shown substantial virus shedding at this time (Garver et al., 2016a; Wessel et al., 2017).

In the third (persistent) phase of infection, viral inclusions within erythrocytes disappear and a marked reduction in viral protein production occurs even though large quantities of genomic PRV material remain associated with the erythrocyte cell fraction (Haatveit et al., 2017; Lund et al., 2017; Polinski et al., 2019). The ability to recapitulate infectious replication of PRV from late stage infections has been readily accomplished by injecting lysed blood cell material into naïve fish (Polinski et al., 2019); however, poor viral transmission has also been demonstrated via cohabitation during this late infectious stage, suggesting natural shedding of virus might be minimal during persistent infections and may even cease entirely over time (Garver et al., 2016a). If heart inflammation occurs, it is typically observed early in the persistent infection phase, although in some instances heart inflammation has occurred just prior to this phase during the peak of infection (Lund et al., 2017; Wessel et al., 2017; Polinski et al., 2019). This inflammation can last for weeks to months depending on a number of factors, but ultimately appears to resolve in all cases even though PRV infections continue to persist (Di Cicco et al., 2017; Lund et al., 2017).

## GENERAL PATHOGENICITY OF PRV

The perceived capacity of PRV to cause disease in many regards closely mirrors that of Avian orthoreovirus (ARV) in poultry. Namely, its impact varies widely from region to region and its ubiquitous nature is often associated with diseases for which a causative link cannot be established (Jones, 2000). It should be noted that in controlled experimental trials, PRV has (as yet) never caused clinical morbidity or mortality in salmon even during extreme blood infections (Garver et al., 2016a; Takano et al., 2016; Wessel et al., 2017; Polinski et al., 2019), nor has it contributed to clinical morbidity or mortality during experimental trials in accompaniment with stressors such as smoltification, viral co-infection, hypoxia, or exhaustive chasing (Garver et al., 2016a; Lund et al., 2016; Polinski et al., 2016; Lund et al., 2017; Zhang et al., 2019). However, all three genogroups of PRV can at the very least contribute to disease states in salmon of variable significance (Olsen et al., 2015; Takano et al., 2016; Wessel et al., 2017; Polinski et al., 2019; Vendramin et al., 2019). Thus, all three genogroups of PRV have pathogenic potential but low virulence due to the inability of extreme systemic infections to cause mortality or morbidity under controlled laboratory conditions.

PRV is typical for a reovirus, but unlike many other viruses, in that it does not directly lyse the cells it infects (Finstad et al., 2014; Wessel et al., 2015; Polinski et al., 2019). Rather, the pathogenic potential of PRV likely stems from the killing of infected cells via an adaptive (T-cell mediated) immune response by the host fish (Mikalsen et al., 2012; Yousaf et al., 2012; Zhang et al., 2019). In other words, PRV itself does not appear to inflict damage to host cells, but if

host immune T-cells develop an ability to recognize PRV as a foreign invader, infected cells become targeted by these sensitized T-cells for destruction. In some instances this appears to results in immune cells targeting infected cardiomyocytes and cardiac epithelial cells such as during heart and skeletal muscle inflammation (HSMI) (Mikalsen et al., 2012). In others instances, infected erythrocytes have been suggested to become targeted for destruction while passing through the liver or spleen such as possibly during Jaundice anemia of Chinook Salmon (*Oncorhynchus tshawytscha*) (Di Cicco et al., 2018). The mechanisms for initiating these adaptive host responses to PRV (if they can be confirmed) are unknown, and it is also unclear why some cell types are more selectively targeted for destruction than others in different instances; e.g., cardiomyocytes and not erythrocytes in Atlantic Salmon even though erythrocytes are the primary cell type infected (Zhang et al., 2019). Recent investigations have suggested that these mechanisms are highly variable with regard to the host species, host strain (possibly even the individual), and to the PRV isolate involved (Polinski et al., 2018; Wessel et al., 2018a). Current knowledge regarding the pathogenic potential of each PRV genogroup is outlined below.

#### PRV-1

At least one purification-based isolation of PRV-1 has been demonstrated to cause severe heart inflammation in farmed Atlantic Salmon of Norway (Wessel et al., 2017) and both PRV-1a and PRV-1b have been physically isolated from HSMI diseased fish in net-pen farm environments [for summary, see (Garver et al., 2016a)]. In Norwegian Atlantic Salmon aquaculture, HSMI is associated with morbidity, lethargy, and occasional mortality; it is considered one of the most significant transmissible diseases affecting industry production (Hjeltnes B et al., 2017).

The inflammation generated during HSMI is likely mediated by an adaptive cytotoxic T-cell response to PRV-1 antigen (Mikalsen et al., 2012). This hypothesis is supported by the increased presence of cytotoxic T-cells in the heart of HSMI diseased fish in accordance with increased transcription of their killing enzymes, e.g., granzyme-A (Mikalsen et al., 2012) and that cytotoxic T-cells are also responsible for reovirus-induced heart inflammation in mammals (London et al., 1990; Gujar et al., 2010). Nevertheless, the clinical severity of HSMI as seen on industry farms in Norway has not been recreated in controlled experimental conditions despite the generation of high-load PRV infections with or without hypoxic stress (Lund et al., 2017; Wessel et al., 2017) – indicating that factors specific to the commercial field environment in Norway contribute to HSMI. This is supported by previous finding that HSMI increases in prevalence with increasing cohort lifespan, infection pressure, cohort size, and local geography (Kristoffersen et al 2013). Heightened disease scenarios are also likely driven in part by host-specific factors as evidenced by the development of a strain of Atlantic Salmon in Norway that had less heart damage and higher survival but were not resistant to PRV infection (AquaGen, 2017; Emilsen et al., 2017); further supporting that host sensitivity to PRV may play a critical role in determining the severity of disease.

In Pacific Canada, PRV-1 has been suggested to be a contributing factor in a jaundice/anemia syndrome of farmed Chinook Salmon (Di Cicco et al., 2018) as well as severe cardiomyopathy in farmed Atlantic Salmon (Di Cicco et al., 2017; Di Cicco et al., 2018). Although it is highly likely that PRV can and occasionally does contribute to both conditions, the role for how or if PRV acts as the etiological mediator of these relatively rare diseases in BC is far from clear. Specifically, neither jaundice/anemia nor severe cardiomyopathy has been successfully transmitted to naive Chinook or Atlantic Salmon in laboratory challenge trials in Pacific Canada despite the successful passage and development of high-load PRV blood infections within both species (Garver et al., 2016b; Polinski et al., 2019). This type of passage experiment is critical

for establishing and identifying pathogenicity of a microbial agent (Fredericks and Relman, 1996), and the lack of virulence demonstrated by high-load PRV infections on these occasions indicates that other critical etiological factors are necessary to establish these disease conditions. This is further supported by the low prevalence of jaundice/anemia or HSML-like cardiopathy compared to the high prevalence of PRV in farmed populations of Chinook and Atlantic Salmon in British Columbia, respectively.

## PRV-2

In Japan, PRV-2 has been shown to be associated with an anemic condition of farmed Coho Salmon known as erythrocytic inclusion body syndrome or EIBS and has caused moderate anemia in Coho Salmon following experimental infection (Takano et al., 2016). Significant mortality has been historically attributed to EIBS in Japan during farming of Coho Salmon (Takahashi et al., 1992); although experimental challenges with PRV-2 have failed to cause mortality (Takano et al., 2016). The mechanisms behind PRV-2 pathogenicity are unknown, but as with PRV-1, factors specific to field environments appear to exacerbate the severity of disease and associated mortality (Takano et al., 2016). It may be hypothesized from work done with PRV-1 that a T-cell mediated sensitivity might be responsible for the anemia observed during EIBS in Japan via a mechanism of targeted destruction of infected erythrocytes as they pass through the liver or spleen. Of particular note in considering PRV-2 relative to other PRV genogroups is the staggering quantity of virus generated during peak infection (approximately one trillion genomic copies per mL blood) in both experimentally and naturally infected fish (Takano et al., 2016). These quantities appear to be 10 to 1,000 times greater than produced during PRV-1 infections of Atlantic Salmon (Garver et al., 2016a; Polinski et al., 2019; Zhang et al., 2019) and at least 1,000 to 10,000 times higher than the most robust PRV-1 infections reported in Pacific Sockeye Salmon (Polinski et al., 2016).

## PRV-3

PRV-3 has been detected in association with an anemic/HSML-like condition in farmed Rainbow Trout in Europe (Olsen et al., 2015) and a jaundice/anemia syndrome in farmed Coho Salmon in Chile (Godoy et al., 2016). PRV-3 was initially thought to also be the causative agent of a proliferative darkening syndrome (PDS) in Brown Trout in central Europe (Kuehn et al., 2018); however, subsequent investigation has demonstrated that PRV-2 is not the causative agent of PDS (Fux et al., 2019). Low to moderate mortality occurs in Rainbow Trout suffering from the anemic/HSML condition (Olsen et al., 2015) although the role that PRV-3 plays in the development of these diseases remains unclear. As for PRV-2, it could be speculated to be driven by cytotoxic T-cell recognition. A laboratory study conducted to assess the pathogenicity of a Norwegian variant of PRV-3, demonstrated that PRV-3 infections of Rainbow Trout were capable of generating heart inflammation yet failed to recreate anemia (Vendramin et al., 2019). Consequently, the anaemia observed in hatchery outbreaks may be due to a secondary factor triggering a more severe disease as is observed in the field (Hauge et al., 2017). Interestingly, exposure of Atlantic Salmon to PRV-3 isolated from Rainbow Trout revealed a capability for the virus to infect both salmonid species, but faster transmission, more notable antiviral response and more prominent heart pathology were observed in Rainbow Trout, suggesting host species-specific factors are important modulators of PRV-3 associated disease (Hauge et al., 2017).

## REGIONAL VARIATIONS IN VIRULENCE (PRV-1)

In Norway, most farmed Atlantic Salmon become PRV positive, but only some develop disease. This does not appear to be dependent on systemic PRV load, and it is not clear why some

farms experience high losses due to HSML while others do not. Nevertheless, clinical outbreaks of HSML in farmed Atlantic Salmon of Norway are reasonably common (Kongtorp et al., 2004a; Kongtorp et al., 2004b; Kongtorp et al., 2006; Palacios et al., 2010), and laboratory challenge trials have demonstrated a clear ability for PRV to cause severe heart lesions (Wessel et al., 2017). Indeed, laboratory challenge trials in Norway routinely generate severe heart lesions in accompaniment with occasional skeletal muscle lesions similar to those observed on HSML diseased salmon farms (Kongtorp et al., 2004b; Kongtorp and Taksdal, 2009; Mikalsen et al., 2012; Finstad et al., 2014; Lund et al., 2017).

In Pacific Canada, there is a strikingly divergent relationship regarding PRV and its association with disease. PRV appears to be highly prevalent in farmed Atlantic Salmon of Pacific Canada (Marty et al., 2015); yet, a clinical outbreak of HSML as described in Norway (Kongtorp et al., 2004a; Kongtorp et al., 2004b) has never been reported. Two subclinical farm-level cases of HSML-like disease have been suggested to date (Di Cicco et al., 2017; Polinski et al., 2019), but unlike in Norway, this disease could not be transmitted to naïve fish in a laboratory setting (Polinski et al., 2019). Indeed, PRV has failed to cause severe heart lesions or any severity of skeletal muscle inflammation following experimental challenge of Atlantic or Pacific salmon in Pacific Canada (Garver et al., 2016a; Polinski et al., 2019; Zhang et al., 2019). Ongoing laboratory investigations directly comparing PRV-1 isolated in both Norway and the Eastern Pacific have also preliminarily identified that the PRV-1 from the Eastern Pacific is of lower virulence to Norwegian Atlantic Salmon (Wessel et al., 2018a).

Host, virus, and environmental factors may all be responsible or contributing factors for this regional altered virulence of PRV. The relative contribution by each of these putative factors is currently unknown; however, there are at least three potentially significant phenotypic dissimilarities between Canadian and Norwegian PRV-1 that have been revealed through laboratory challenge trials (Figure 2). First, despite the similarity of Pacific Canada PRV and Norwegian PRV to produce high load viremia, Pacific Canada PRV remains absent from the plasma (Polinski et al., 2019) while Norwegian PRV can be detected at high loads in the plasma for up to six weeks following infection (Finstad et al., 2014; Wessel et al., 2017). Second, there is a considerable difference in scale regarding host recognition of PRV. Although direct comparisons between Canadian and Norwegian studies are limited, mean systemic and heart-specific antiviral responses increased no more than fivefold in Pacific Canada studies (Garver et al., 2016a; Polinski et al., 2019; Zhang et al., 2019) whereas in Norwegian challenges these genes increased 10-50 fold in the blood (Haatveit et al., 2017; Wessel et al., 2017) and more than 100 fold in the heart (Mikalsen et al., 2012). The comparative lack of antiviral response to Pacific Canada PRV compared to Norwegian PRV is further supported by the relative protection PRV has afforded fish challenged with a secondary virus (IHNV) in Norway (Vendramin et al., 2018) but not in Pacific Canada (Polinski et al., 2016). Lastly, in addition to the discrepancies concerning the severity of heart inflammation outlined above, the timing of PRV associated heart inflammation is also different between challenges conducted with PRV from these two countries. Specifically, by either injection or cohabitation exposure of PRV, heart inflammation (prevalence and severity) in Norwegian studies consistently begins around the time of peak systemic PRV load, reaches high severity 1-2 weeks later, and thereafter diminishes (Lund et al., 2017; Wessel et al., 2017). In contrast, increased prevalence of heart inflammation in Pacific Canada challenge trials did not occur until approximately 4 weeks after peak PRV systemic loads were reached and maintained high prevalence (although not severity) for prolonged periods of greater than 6-7 weeks (Polinski et al., 2019; Zhang et al., 2019). All challenges were conducted at approximately the same temperature ((10-12°C).

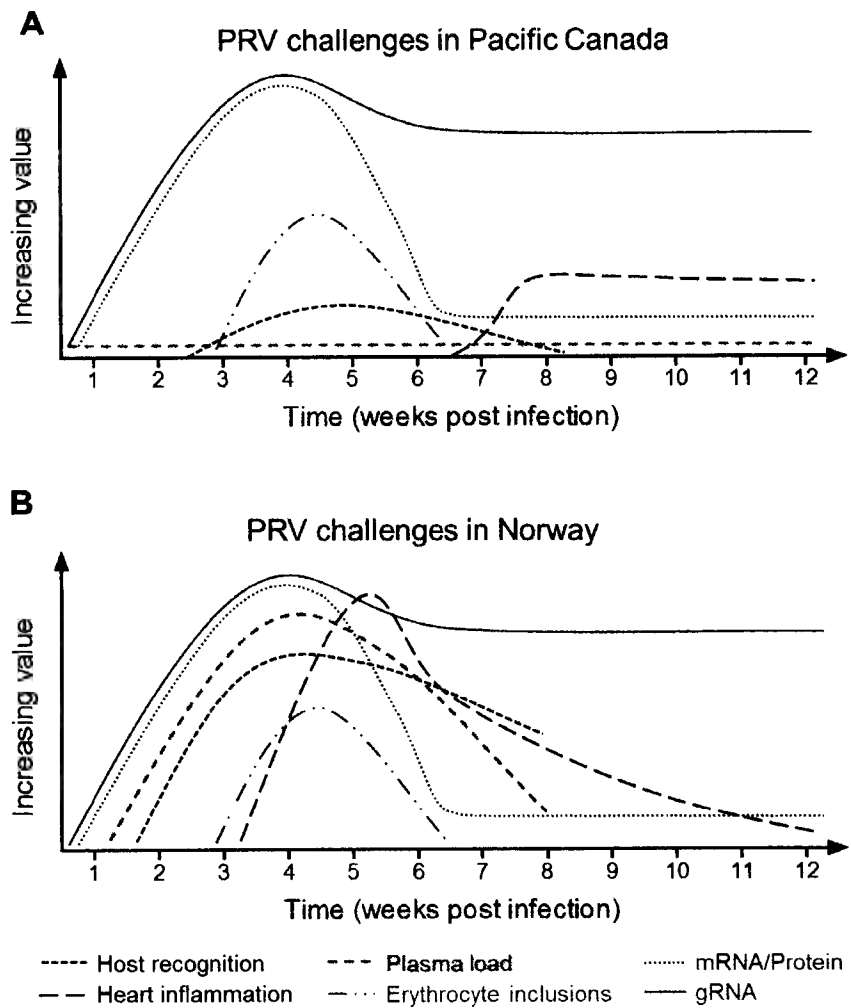


Figure 2. Contrast summary for general trends in PRV phenotypic infection dynamics between Norway and Canada laboratory challenge of Atlantic Salmon (taken from Polinski et al., 2019). In comparing challenge trials conducted in (A) Pacific Canada (Garver et al., 2016a; Polinski et al., 2019; Zhang et al., 2019) with results from similar challenge trials conducted in (B) Norway (Mikalsen et al., 2012; Finstad et al., 2014; Haatveit et al., 2017; Wessel et al., 2017). Note that some variance in experimental design exists between these experiments.

TRANSMISSION DYNAMICS (PRV-1)

Routes of Entry

PRV has been demonstrated to spread horizontally (from fish to fish) during laboratory cohabitation studies where PRV infections become evident in 100% of naïve fish (Garver et al., 2016a; Wessel et al., 2017). The route by which PRV enters naïve hosts remains unclear; however, fecal-oral transmission is a hallmark of many reoviruses and the presence of PRV-1 in feces of infected fish (Hauge et al., 2016) coupled with the demonstrated ability of PRV to infect naïve fish via anal intubation (Hauge et al., 2016) suggests fecal-oral transmission is at least

one likely route for natural PRV entry. Experimental studies have also generated PRV infections following waterborne immersion (Kvamme et al., 2018). Given that direct horizontal transmission of PRV can readily be accomplished, vector-mediated transmission (e.g., via a multicellular parasite) would present an unnecessary step in spreading PRV. Currently there is no evidence to suggest a vector is needed for PRV transmission.

Although the primary mode of PRV transmission is almost certainly horizontal, it is probable, given the systemic nature of PRV infections, that PRV contamination of sexual fluids permits the potential for egg-associated vertical (from parent to egg) transmission. Fish in freshwater hatcheries in both North America and Europe (Lovoll et al., 2012; Polinski et al., 2019) have become infected presumably via this method; however, a study following a population of Norwegian Atlantic Salmon brood fish and progeny from 2008 to 2011 found that PRV was not isolated in or on eggs collected from infected brood fish following a Buffodine® disinfection treatment (Wiik-Nielsen et al., 2012). Similarly, PRV could not be detected in juvenile Atlantic Salmon reared from iodine disinfected eggs at a commercial freshwater facility in British Columbia even though the Atlantic Salmon brood fish supplying the facility carried high systemic RNA loads of PRV (Polinski & Garver, unpublished data). As iodine disinfects egg surfaces but not the internal yolk or embryo, this data suggests that infectious PRV is not carried internally within fertilized eggs of salmon and that, if PRV is present on egg surfaces, iodine-based disinfection has the potential to be used to block infectious PRV transmission from parent to offspring.

## Shedding

PRV infected salmon are considered a main transmission source of virus; yet it remains unknown as to how long and at what rates PRV is shed from an infected fish. Cohabitation studies where naïve salmon were introduced at different stages of PRV infection revealed that Atlantic Salmon recently infected with PRV were capable of transmitting virus but those in persistent stages of infection had reduced or ineffectual transmission to the naïve cohabitants (Garver et al., 2016a; Polinski et al., 2019). Therefore, it is inaccurate to presume that all PRV infected Atlantic Salmon are equally contagious and are likely to transmit virus and it remains unknown as to what factors may impact virus shedding. Laboratory studies in Pacific Canada have demonstrated that Atlantic Salmon were highly infectious after 4-6 weeks of becoming infected with PRV (Garver et al., 2016a) but horizontal transmission was reduced by 15 weeks (Polinski et al., 2019) and could not be accomplished after 44 weeks despite the ongoing persistence of PRV (Garver et al., 2016a). Based on these studies, it is hypothesized that natural horizontal transmission primarily occurs between 3-15 weeks following infection, after which the potential for natural shedding becomes severely reduced (Polinski et al., 2019).

## Environmental stability

It can be presumed that PRV maintains at least a minimum capacity to survive in water, as successful waterborne transmission has been demonstrated experimentally. Yet, the extent to which PRV can remain infectious in the natural marine environment remains unknown. Many environmental factors such as sunlight, organic load, and indigenous microbial populations can adversely affect virus stability to varying degrees dependent upon virus type (Pinon and Viallette, 2018). For instance, viruses surrounded with an envelope are generally more easily rendered inactive than viruses without an envelope (Fitzgibbon and Sagripanti, 2008). Being free of an envelope, PRV could be expected to have greater stability than, for example, the envelope containing aquatic virus infectious hematopoietic necrosis virus (IHNV) which showed markedly reduced infectivity within hours of being held in natural seawater (Garver et al., 2013).

In contrast, infectious pancreatic necrosis virus (IPNV), a nonenveloped aquatic fish virus, requires days to lose infectivity in estuarine water (salinity 11.5 ppt) (Toranzo et al., 1983). However, due to the fact that decay rates are highly conditional upon virus and environmental factors, survival studies specific to PRV are required to accurately define its duration of infectivity in seawater. To date, such studies have not been undertaken due to the lack of conventional culture methodologies to conveniently monitor and evaluate the infectivity of PRV. Furthermore, suitable proxy data is unavailable as viral stability measurements from culturable surrogates such as Chum Salmon aquareovirus, that shares both structural and genomic similarities with PRV, have not been performed.

### Infectious potential

Preliminary evidence using PRV-1 from Pacific Canada suggests that  $\leq 10^3$  PRV particles are sufficient to initiate infection by intra-peritoneal injection in Atlantic Salmon (M. Polinski, unpublished data). The minimum dose required to establish infection by immersion or ingestion is unknown, but the route of virus exposure, the host condition, stock, and species involved are all likely to play a role in the infectious potential of PRV. For example, Sockeye Salmon have exhibited detectable levels of PRV in blood as early as five days post intra-peritoneal injection (Polinski et al., 2016) while Sockeye continually cohabitated with PRV infected Atlantic Salmon did not acquire PRV blood infections until the 4<sup>th</sup> week of cohabitation (Garver et al., 2016a). Further, sentinel Sockeye Salmon showed substantially lower prevalence and intensity of PRV infections than in sentinel Atlantic Salmon of an equivalent exposure group after 4 weeks of cohabitation (Garver et al., 2016a), indicating that Sockeye Salmon are less susceptible to PRV than Atlantic Salmon and may require a more lengthy exposure period or dose to become infected. For newly smolted Pink Salmon (*Onchorhynchus gorbuscha*) (1 g), waterborne exposure to either 100 or 1,000 purified PRV particles per mL for one hour was insufficient to initiate infection (n=20) while an equivalent dose of 1,000 purified particles administered via intra-peritoneal injection established PRV infection in 90% of fish (n=10), suggesting a low susceptibility of Pink Salmon to waterborne infection (Richard, Polinski, and Garver, unpublished data). Refractivity to PRV-1 by immersion has also been preliminarily demonstrated in Sea Trout (*Salmo trutta*) relative to Atlantic Salmon in Norway (Kvamme et al., 2018). It is currently unknown if these reduced susceptibilities are dose and/or duration dependent.

### Farmed-to-wild salmon transmission

Given the linkage with PRV to HSMI in Norwegian Atlantic Salmon farming, investigations have been conducted in Norway to evaluate the transmission of HSMI and PRV between neighboring farms and to wild fish populations. Sequence comparisons of PRV variants collected from farm and wild salmon in Norway revealed that PRV genotypes are similar regardless of host origin, suggesting that virus exchange is occurring between wild and farmed populations in Norway (Garseth et al., 2013; Madhun et al., 2018). However, neither the directionality nor the mechanism(s) responsible for exchanging PRV between farmed and wild populations are currently known. It has been postulated that interactions between wild and escaped farmed salmon, specifically when wild salmon migrate through aquaculture areas, may serve as potential mechanisms of virus perpetuation (Garseth et al., 2013). Nevertheless, comparisons of PRV prevalence in wild adult salmon from regions of northern Norway with differing farming intensity and disease frequency showed no association between salmon farming and the prevalence of PRV infection in wild salmon (Madhun et al., 2018).



In western North America, the high genome homology between PRV-1 isolates of farmed and wild salmon (Siah et al., 2015) suggests the presence of a common reservoir and/or exchange of virus between wild and farmed populations. Yet the contribution of salmon farms to potentially exchange PRV with wild fish is unclear. One study has hypothesized salmon farms may influence PRV prevalence in wild Pacific salmon after identifying a higher prevalence of PRV in wild salmon with a “high” exposure probability to salmon farms than in fish sampled from “low” farm-exposure regions (Morton et al., 2017); although it must be noted that the categorization for low and high farm exposure used in this study is highly speculative. In contrast, a study that compared detection of PRV in Coho Salmon from Alaska (an area devoid of open net pen salmon aquaculture) to Coho Salmon from British Columbia (where salmon farms are present) identified no significant difference in PRV detection prevalence, suggesting salmon farming was contributing negligibly to PRV prevalence in these wild Coho stocks (Marty et al., 2015). Chinook Salmon also screened for PRV in Alaska (Purcell et al., 2018) similarly showed analogous detection prevalence and stock variability for PRV detection to Chinook Salmon of British Columbia (Marty et al., 2015) (Table 2); also suggesting that salmon farms are having minimal direct impacts to PRV prevalence in Chinook of the Eastern Pacific. Undoubtedly a multitude of factors are responsible for influencing PRV prevalence in wild salmon, which is clearly evident by the fact that PRV detection varies considerably across host species and even between cohorts of a particular species (Purcell et al., 2018). Consequently, longer time scale monitoring efforts in conjunction with molecular epidemiology studies are needed to fully appreciate the drivers of PRV infection in salmon population of western North America.

## PREVALENCE IN WESTERN NORTH AMERICA

Molecular diagnostic screening has been applied in numerous surveillance programs that have identified the presence of PRV among farmed and wild salmon collected over the geographic range spanning Alaska to Washington. Analyses of archived salmon samples from 1974-2008 from British Columbia also revealed a long-term and common presence of PRV-1 in the Eastern Pacific with positive detections identified in samples dating back to 1987 and possibly as early as 1977 (Marty et al., 2015). Both farmed and wild fish stocks have been shown to become infected.

### Farmed Atlantic Salmon

Once PRV becomes present on a salmon farm, it is expected to reach 100% prevalence within the population (Di Cicco et al., 2017; Polinski et al., 2019). In a temporal study of PRV at one Atlantic Salmon farm site in British Columbia, PRV was first detected 3 to 4 months following seawater entry and peaked at 100% several months later (Di Cicco et al., 2017). A second study also identified 100% PRV prevalence at a different British Columbia Atlantic Salmon farm site after fish had spent 3 months at sea (Polinski et al., 2019). More recently, a sampling survey of dead or dying fish collected in all aquaculture zones of BC demonstrated that time-at-sea was a significant predictor for the detection of PRV in Atlantic Salmon with prevalence increasing up to 18 month post seawater entry and declining thereafter (Laurin et al., 2019). Additionally, current ongoing research examining PRV prevalence on 13 Atlantic Salmon farms spread across British Columbia found that fish at all 13 sites became infected with PRV with a general onset within 100 and 200 day post seawater entry that was independent of location or time of stocking. Further, following initial infection, all 13 farms reached 100% infection prevalence within 100 days of first detection (Polinski and Garver, unpublished data).

**523 Wild Pacific salmon**

524 Either through experimental or natural infection, all five species of North American Pacific  
525 salmon have been presumed to be capable of supporting PRV infections as evidenced by the  
526 internal detection of PRV RNA in all five species (Table 1); however, surveys of wild Pacific  
527 salmon demonstrate that PRV detection can vary dramatically between species and stock.  
528 Across multiple independent surveys of Pacific salmon and trout, PRV was consistently  
529 detected in Chinook and Coho salmon as compared to Chum (*O. keta*), Pink, Sockeye, and  
530 steelhead Trout (*O. mykiss*). Collectively across the studies, while understanding that there are  
531 no reported test performance characteristics for the various studies and that they differ in  
532 sampling protocols, analytical techniques and quality control stringencies, face value detection  
533 prevalence for Chinook and Coho salmon identified within these studies reached approximately  
534 6% and 9%, respectively, while PRV detection prevalence in Pink Salmon remained below 4%,  
535 Sockeye around 1.4%, and Chum as well as steelhead less than 1% (Table 2).

Table 2. PRV-1 prevalence in North American Pacific salmon and trout species sampled in Alaska, British Columbia, and Washington. Numbers in parentheses represent the PRV positive fish per total number of fish sampled. Results are reported at face value without consideration of differences in sampling or assay design.

Species	PRV-1 surveillance studies						Overall prevalence
	(Marty et al., 2015)	(Purcell et al., 2018)	(Morton et al., 2017)	S. Johnson unpublished	Fluidigm BioMark™ assay <sup>a</sup>	Unpublished student theses <sup>b</sup>	
Sockeye Salmon	0.3% (1/371)	0.0% (0/394)	9.3% (21/225)	0.0% (0/717)	1.6% (67/4215)	1.0% (8/771)	1.4% (97/6693)
Chinook Salmon	8.8% (6/68)	4.0% (19/480)	34.3% (34/99)	4.4% (54/1232)	2.8% (9/325)	2.4% (1/41)	5.5% (123/2245)
Coho Salmon	7.6% (9/118)	11.8% (56/473)	26.1% (18/69)	4.5% (16/356)	1.7% (1/61)	--	9.3% (100/1077)
Pink Salmon	0.0% (0/313)	0.4% (1/243)	25.0% (27/108)	0.0% (0/70)	--	-- <sup>1</sup>	3.8% (28/734)
Chum Salmon	0.0% (0/101)	0.0% (0/287)	7.5% (5/67)	0.0% (0/135)	--	--	0.8% (5/590)
Steelhead Trout	--	0.3% (1/375)	28.6% (4/14)	--	--	1.0% (3/303)	0.9% (5/553)
Cutthroat Trout	--	--	50.0% (8/16)	--	--	trout combined	--
Dolly Varden Trout	--	--	10.0% (1/10)	--	--	--	--

<sup>a</sup> (Jeffries et al., 2014; Miller et al., 2014; Bass et al., 2017; Teffer et al., 2017; Nekouei et al., 2018; Teffer et al., 2018; Thakur et al., 2019)

<sup>b</sup> (Furey, 2016; Healy, 2017; Hrushowy, 2018; Stevenson, 2018)

Specific to Sockeye Salmon, 12 independent studies cumulatively indicate that the majority of samples positive for PRV nucleic acid were collected from returning adults (Table 3). A cumulative 0.3% (12/3911) of fry and juvenile fish were positive for PRV whereas 2.9% (85/2912) of returning adults were positive. This data also suggests that most PRV infections occurred at sea. PRV was detected on or in out-migrating smolts collected at the mouth of Queen Charlotte Strait and within the southern Queen Charlotte Sound (after presumed northward migration through the Discovery Islands/Johnson Strait) at a prevalence of 0.8% (7/833), whereas fry and parr had a nominally lower prevalence of 0.4% (4/1072) in freshwater, with most detections (3) occurring in a population of fry from Oweekeno Lake that is not associated with the Fraser River (Table 3). Similarly, the majority of PRV detections in adult Sockeye Salmon (63/85 total positives) occurred in one study which screened gill biopsies of returning adult fish migrating southwards through the Johnston Strait/Discovery Islands (Miller et al., 2014). Interestingly, liver samples taken at the same time of gill biopsies as well as subsequently in the Fraser River were negative for PRV; suggesting that the PRV on or in the gill tissues of these fish did not represent systemic infections nor did systemic infections likely develop before returning fish reached their spawning grounds.

Within the Fraser River, PRV has been detected in at least five stocks of Sockeye Salmon (Table 4), although sampling for many stocks has been limited and the single Nadina River sample positive for PRV nucleic acid was considered questionable by the authors (Marty et al., 2015). Further, it should again be noted that 63/68 positive PRV detections occurred as a result of gill biopsies taken from returning adults passing through the Johnstone Strait/Discovery Islands which did not appear to develop systemic infections (Miller et al., 2014).

564 Table 3. Detection of PRV in Sockeye Salmon of Alaska, British Columbia, and Washington by life stage.  
 565 Numbers in parentheses represent the PRV positive fish per total number of fish sampled. Cumulative  
 566 detection specific to Fraser River Sockeye Salmon (FRSS) stocks (where identified) is also presented; the  
 567 29 adult fish sampled in saltwater by Morton et al. are of unknown (possibly Fraser River) origin but not  
 568 incorporated into the FRSS summary.

Data Source	Sockeye Salmon PRV prevalence				
	Fry	Parr/smolt		Adults	
	Freshwater	Freshwater	Saltwater	Saltwater	Freshwater
Marty et al. (2015)	--	0/30	--	--	1/341
Purcell et al. (2018)	--	--	--	--	0/394
Johnson (unpublished)	--	0/344	0/373	--	--
Morton et al. (2017)	--	1/1	3/90	0/29	17/105
Miller et al. (2014)	--	--	1/165	64 <sup>1</sup> /531	1/498
Teffer et al. (2017)	--	--	--	--	0/112
Thakur et al. (2019)	--	--	--	--	0/652
Nekouei et al. (2018)	--	0/896	1/1110	--	--
Jeffries et al. (2014)	--	0/228	--	--	0/23
Stevenson (2018)	--	0/300	--	--	--
Furey (2016)	--	0/80	--	--	--
Hrushowy (2018)	3/89	--	3/205	--	2/97
<b>Totals</b>	<b>3.4% (3/89)</b>	<b>0.1% (1/1879)</b>	<b>0.4% (8/1943)</b>	<b>11.4% (64/560)</b>	<b>0.9% (21/2222)</b>
<b>Totals (FRSS only)</b>	<b>--</b>	<b>0.1% (1/1505)</b>	<b>0.2% (2/1258)</b>	<b>12.1% (64/531)</b>	<b>1.3% (19/1431)</b>

569 <sup>1</sup>63/155 detections of PRV from gill biopsies but 0/57 detections in liver tissues collected at same location

Table 4. Distribution of PRV detection across Fraser River Sockeye Salmon stocks (Jeffries et al., 2014; Miller et al., 2014; Marty et al., 2015; Furey, 2016; Morton et al., 2017; Teffer et al., 2017; Nekouei et al., 2018; Stevenson, 2018).

Stock screened for PRV	PRV screening results	
	Juveniles	Adults
Bowron	0/9	--
Cultus	1/62	--
Weaver	0/8	--
Portage	0/35	--
Early Stuart, Late Stuart & Misc. <sup>1</sup>	0/4	1/191
Quesnel	0/22	0/297
Horsefly	0/148	--
Mitchell	0/119	--
Blue Lead	0/1	--
Wasko-Roaring	0/16	--
Nahatlatch River	0/16	--
Fennell	0/1	--
Thompson	0/75	--
Raft	0/18	--
Upper Barrier	0/3	--
Birkenhead	0/77	0/11
Scotch	0/72	0/8
Seymour	0/134	--
Adams	1/370	0/2
Shuswap	0/398	49/304
Eagle	0/6	--
Little	0/5	--
Nadina	0/60	1 <sup>2</sup> /60
Dolly Varden	0/86	--
Chilliwack Lake	0/34	--
Stellako	0/137	0/10
Gates	0/65	0/19
Big Silver	0/4	--
Pitt	0/79	--
Harrison	--	0/103
Chilko	0/1018	15/250

<sup>1</sup> This includes juvenile fish sampled from Sandpoint Creek, Five Mile Creek, Middle River, and Dust-Sinta Creek (n=1 per stock).

<sup>2</sup> Positive detection of PRV nucleic acid in only one of two technical replicates which was noted as inconclusive by the authors (Marty et al., 2015).

## CARDIOPATHY OF SALMON

### CAUSATIVE FACTORS

Cardiopathy refers to diseases of the heart that affects contractive functions and decreased capacity to circulate blood. These diseases have many causes and, in association with the global production of farmed salmon, a variety of cardiopathies have been described. Some occur as a result of non-transmissible conditions such as during cardiac remodeling and expansion due to chronic hypoxia stress (Simonot and Farrell, 2007) or as a result of congenital mutation (Becker et al., 2011). However, cardiopathy can also occur due to infectious and transmissible microbes. Specific to salmon, at least eight infectious agents are known to cause cardiopathic disease, although high-load systemic infections of virtually any moderately virulent pathogen has the potential to inflict damage to heart tissues:

- *Renibacterium salmoninarum* (Bruno, 1986)
- *Piscirickettsia salmonis* (Olsen et al., 1997)
- *Kudoa thyrsites* (Moran et al., 1999)
- *Ichthyophonus hoferi* (Kocan et al., 2006)
- *Yersinia ruckeri* (Rucker, 1966)
- Salmonid alpha virus (SAV) (Wiik-Nielsen et al., 2016)
- Piscine myocarditis virus (PCMV) (Haugland et al., 2011)
- Piscine orthoreovirus (PRV) (Wessel et al., 2017)

Of these, six are endemic to British Columbia: *I. hoferi*, *K. thyrsites*, *P. salmonis*, *R. salmoninarum*, *Y. ruckeri* and PRV. In the event that the causative agent of a heart disease is not clearly identifiable, a diagnosis of idiopathic cardiopathy is assigned.

### PREVALENCE AND IMPACT IN BRITISH COLUMBIA

#### General cardiopathy

Mild cardiopathy is prevalent in farmed salmon of British Columbia; however, severe cardiopathy impairing heart function is rare. Between 2006 and 2018, the Fish Health Auditing and Surveillance Program (FHASP) conducted by DFO Aquaculture Management Division has evaluated all major organs of nearly 6,000 Atlantic and 800 Pacific (majority Chinook; some Coho) salmon net-pen farming mortalities by histopathology including heart tissues. Mild to moderate cardiopathy occurred in 61% of Atlantic and 41% of Pacific salmon mortalities sampled during this period. However, this cardiopathy, mainly epi- and endocarditis, does not compromise heart or respiratory function (Lund et al., 2017; Zhang et al., 2019) and is not expected to adversely affect salmon health. Moderate to severe cardiopathy with a putative ability to negatively affect heart functioning was diagnosed in 7% and 3% of Atlantic and Pacific salmon mortalities, respectively. The severity was sufficient to be suggested as a putative cause or contributing factor to death in less than 3% of both Atlantic and Pacific salmon species (Figure 3). These percentages are representative of sites specific to the Discovery Islands region and are consistent with other independent studies which have corroborated the relatively widespread occurrence of generally mild cardiopathy in British Columbia salmon with little evidence for its contribution to morbidity or mortality over the past decade (Marty et al., 2015; Di

## PRV and Associated Disease

DRAFT (Do Not Cite or Distribute)

Cicco et al., 2017). This is also consistent with previous diagnoses of prevalence for severe cardiopathy in farmed salmon from the early 1990's (Brackett et al., 1990; Brackett et al., 1991; Brackett and Newbound, 1992; Brackett et al., 1992); suggesting that cardiopathy has likely caused or contributed to less than 0.4% cumulative mortality in farmed salmon in BC over the past 25 years. The proportion of this cardiopathy that is attributable to infectious diseases and specifically PRV is unknown, although multiple transmissible and non-transmissible factors are indicated to be involved in addition to PRV (Figure 3).

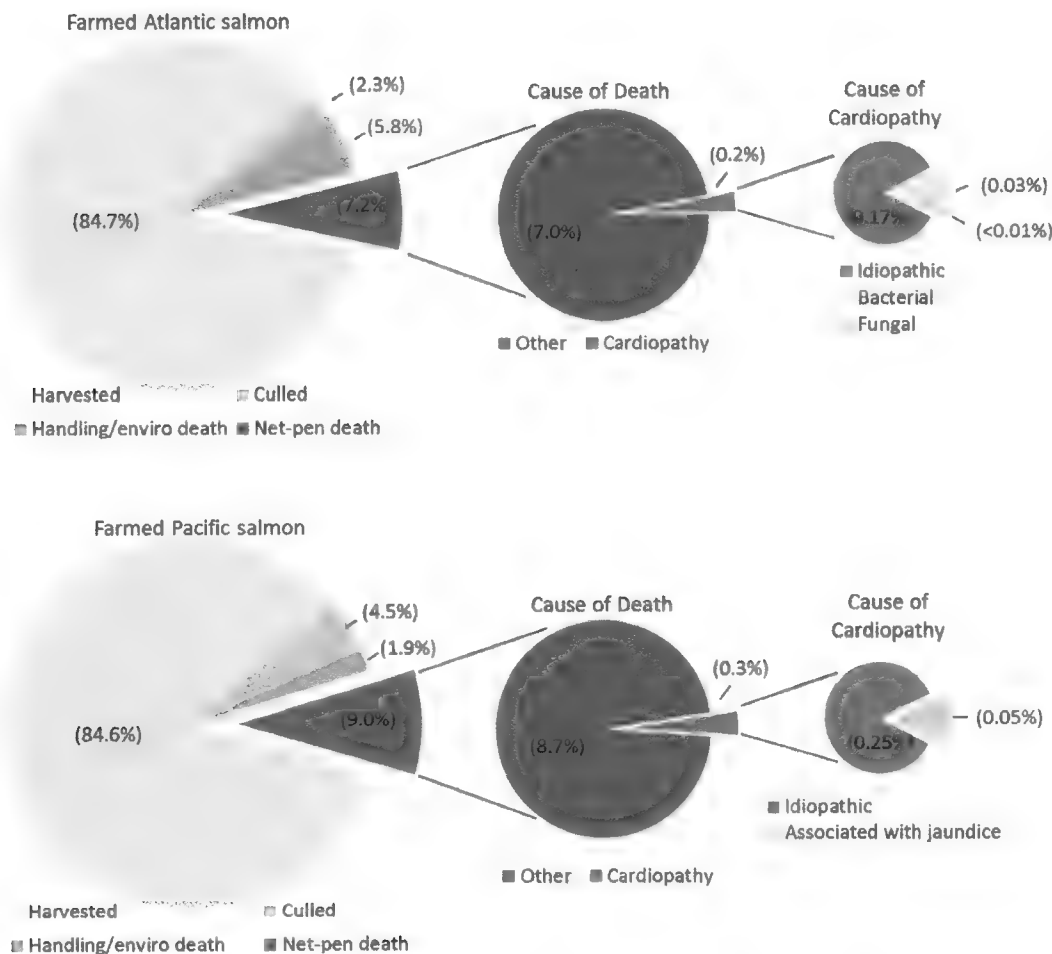


Figure 3. Cardiopathy as a marker of death in farmed salmon of British Columbia. Cumulative percent mortality of stocked fish per annum presented in left pie charts are extrapolated from the mean monthly mortalities reported across salmon farming industries between 2012-2018. Putative cause of death diagnoses from net-pen mortalities not associated with culling, handling, or environmental causes (e.g., low dissolved oxygen) is presented in the center pie charts based on FHASP data collected between 2006 and 2018. The putative causes of cardiopathy in these instances are presented in the right pie charts. All percentages are relative to total number of net-pen Atlantic or Pacific salmon stocked per annum during this time period.



The prevalence of cardiopathy in wild Pacific salmon is relatively unknown; however, a survey of 204 wild salmon of British Columbia in 2013 that included Pink, Chum, Chinook, Coho, and Sockeye salmon diagnosed mild cardiopathy in 12 fish (5.9%) but failed to identify significant (severe) cardiopathic disease (Marty et al., 2015). Similarly, severe cardiopathy that occurs in farmed Atlantic Salmon in Norway (e.g., HSMI, CMS and PD) has not been detected in wild Atlantic Salmon (Garseth et al., 2013), suggesting environmental components specific to intensive culture likely enhance the prevalence and severity of cardiopathy in salmon.

## HSMI

The term HSMI, although foundationally descriptive, has evolved considerably in meaning over the past decade. Before a causative agent was known, the original diagnosis of HSMI was founded on a set of distinct clinical disease characteristics in Norwegian Atlantic Salmon farms during episodes of morbidity and/or mortality for which histopathology was used to confirm and differentiate this condition from other similar diseases; e.g., pancreatic disease or cardiomyopathy syndrome. By this original case definition, HSMI has never been reported in British Columbia:

*"Affected fish are anorexic and display abnormal swimming behaviour. Autopsy findings typically include a pale heart, yellow liver, ascites, swollen spleen and petechiae in the perivisceral fat. Diagnosis of HSMI is presently based on histological examination. HSMI is characterised by extensive panmyocarditis and myositis, particularly involving red skeletal muscle. Morbidity may be very high, while mortalities are variable and may reach 20% in affected cages."* (Kongtorp et al., 2004a).

Following the discovery of PRV in association with HSMI in Norway in 2010 (Palacios et al., 2010) and the subsequent demonstrated ability for PRV to cause severe heart inflammation (Wessel et al., 2017), the diagnosis for HSMI, although still exclusively based on histopathology, is generally accepted to be initiated by PRV. Many subclinical infections of HSMI have now been diagnosed in Norway, some even without the evidence of skeletal muscle inflammation, and although environmental and/or host contributing factors may explain the often exacerbated severity of HSMI in a field relative to laboratory setting, PRV appears to be the sole infectious agent associated with the unique set of histopathological criteria that defines HSMI in Norway (Palacios et al., 2010; Wiik-Nielsen et al., 2016; Wessel et al., 2017). To our knowledge, HSMI has never been used to classify a disease state in Norway where PRV has been confirmed to be absent.

Two recent studies from Pacific Canada have also used the term HSMI to classify subclinical heart disease of farmed Atlantic Salmon based on histopathology in accordance with their own definitions similar to those previously reported in Norway – namely, moderate to severe heart inflammation sometimes accompanied by skeletal muscle inflammation (Di Cicco et al., 2017; Di Cicco et al., 2018). Although the presumed commonality for the heart and skeletal muscle lesions in these Canadian studies relative to HSMI diagnosed in Norway is the causation by PRV, there is far less evidence in Canada to support that PRV is indeed the key component for initiating this relatively rare disease state; particularly given that these modified definitions have occasionally been observed in the absence of PRV (Marty and Bidulka, 2013; Di Cicco et al., 2018). Consequently, if HSMI diagnosis is based solely on histopathological heart lesions which can occur in the absence of PRV, then PRV cannot be assumed to be *the* causative agent of the disease, but rather one of multiple stand-alone or synergistic putative factors. Thus, if using the definition proposed by Di Cicco et al. (2017), HSMI in British Columbia can likely be used interchangeably with the terms 'moderate to severe cardiopathy' or 'idiopathic cardiopathy' as

described above. Throughout this review, we use the term HSMI only in cases which fit those described by Wiik-Nielsen et al (2016) where PRV appears to be the most likely primary causative factor.

## ANEMIA OF SALMON

### CAUSATIVE FACTORS

Anemia is a condition marked by a deficiency in red blood cells and/or haemoglobin that results in a reduced ability for blood to transport oxygen. Many factors can cause or contribute to anemia in fish including nutrient deficiencies, toxic agents and infectious pathogens (Witeska, 2015). Specific to salmon, at least eight pathogenic organisms (including viruses, bacteria, and external parasites) are known to directly or indirectly contribute to anemia although this is almost certainly not a comprehensive list:

- Infectious salmon anemia virus (ISAV) (McBeath et al., 2015)
- Infectious hematopoietic necrosis virus (IHNV) (Amend and Smith, 1975)
- Piscine orthoreovirus (PRV) (Takano et al., 2016)
- *Aeromonas* sp. (Řehulka, 2002)
- *Flavobacterium columnare* (Řehulka and Minařík, 2007)
- *Vibrio anguillarum* (Harbell et al., 1979)
- *Ichthyophthirius multifiliis* (Abdel-Hafez et al., 2014)
- *Tetracapsuloides bryosalmonae* (Hoffmann and Lommel, 1984)

Of these, there are five relevant to salmon of British Columbia that reside in saltwater: IHNV, PRV, *Aeromonas* sp, *F. columnare* and *V. anguillarum*. However, although PRV is listed here, it must be noted that the only PRV isolate to induce anemia in salmon through experimental infection to date is that of PRV-2 from Japan (Takano et al., 2016).

It also must be acknowledged that the measures used to assess anemia (erythrocyte count, haemoglobin concentration and hematocrit) are at best indirect measures because they do not necessarily imply a lack of sufficient oxygen delivery. This is highly important when considering that, unlike for mammals, these measures can fluctuate substantially in healthy populations of fish. In salmon, the packed erythrocyte volume (hematocrit) can vary as much as 40% between individuals within a single cohort population (i.e., hematocrit ranging from 40-65% of total blood volume) without significant correlation to respiratory functioning (Zhang et al., 2019). For salmon, it has been suggested that functional anemia occurs when hematocrit drops below approximately 20-25% total blood volume (Simonot and Farrell, 2007; Clauss et al., 2008), although this estimate will likely vary depending on a variety of environmental and host-specific factors. Thus, clinical symptoms such as lethargy or other signs of morbidity and/or mortality are important characteristics for identifying fish which are truly anemic (i.e., have a loss in respiratory function) relative to those which may have a reduced hematocrit or haemoglobin relative to what is 'typical' for the species but remain physiologically uncompromised.

**IMPACT AND PREVALENCE IN BRITISH COLUMBIA**

Unexplained anemia (i.e., with the potential to be caused by an unknown pathogen such as PRV) is rarely documented in salmon of British Columbia. There were no veterinary diagnoses indicative of anemia based on excessive internal organ pallor or jaundice in farmed Atlantic Salmon made as part of the FHASP within DFO between 2011 and 2017 that included 663 site visits and 4,344 sampled specimen carcasses. There were also no diagnoses of anemia in farmed Coho Salmon during this period (17 site audits involving 75 collected specimens), although it should be noted that subclinical anemia would likely be missed by visual inspection of moribund or recently deceased carcasses. In farmed Chinook Salmon, a condition referred to as Jaundice Syndrome was diagnosed by the FHASP veterinarian for 7 out of 479 carcasses (1.5%) in 5 of 95 site audits during this seven year period. Jaundice Syndrome is characterized by yellow discoloration of the skin resulting from excessive bilirubin in the blood due to red blood cell breakdown. Substantial red blood cell breakdown is needed to cause jaundice and can therefore be used as a proxy for identifying current or recent anemia. The prevalence of jaundice/anemia reported during the FHASP is similar to that reported previously in farmed Chinook Salmon of BC (< 1.5% jaundice-associated mortality per production cycle) that appears to have a seasonal (cold water temperature) component (Garver et al., 2016b). This seasonality is at least partially substantiated by a focused study involving 210 FHASP samples collected from Chinook Salmon in 2011-2013 by Di Cicco et al. (2018), where the authors noted what they classified as jaundice syndrome based on their own definitions using histopathology (a more liberal classification than previously used by government or industry veterinarians) in 14 fish (6.7%) that was restricted to two specific sampling events. The occurrence of unexplained anemia in wild Chinook Salmon or any other Pacific salmon species in British Columbia is unknown.

**RELATIONSHIP OF PRV-1 AND DISEASE IN BRITISH COLUMBIA**

Infectious disease investigations often draw on a multi-varied approach of field studies, epidemiological analyses, and laboratory experimentation to understand the biology and disease causing potential of an infectious agent. Ultimately the proof of disease causation rests on the concordance of scientific evidence, with one of the key components in establishing a causative link between agent and disease being the reproduction of disease when the purified agent is introduced into a naïve host. For disease investigations of PRV in Norway, Denmark and Japan, laboratory experimentation has demonstrated that specific disease conditions could be reproduced when either Atlantic or Pacific salmon species received one of the three genetic types of PRV (Takano et al., 2016; Wessel et al., 2017; Vendramin et al., 2019). These studies not only demonstrated specific PRV types to have disease causing potential but also importantly established that laboratory environments are a suitable surrogate for investigating PRV associated diseases. Consequently, to investigate the relationship of PRV-1 and disease in salmon of British Columbia, laboratory and field based studies were conducted similar to those carried out in countries where an etiological link of PRV with disease has been established. This section provides an overview of the studies investigating PRV-1 and its link to disease in British Columbia.

**ATLANTIC SALMON**

Experimental challenge trials using PRV-1 in Pacific Canada Atlantic Salmon have resulted in extreme PRV blood infections, but either failed to generate notable pathology (Garver et al., 2016a) or induced only minor to moderate heart inflammation – specifically, epi- and

endocarditis (Polinski et al., 2019; Zhang et al., 2019). Further, the increased prevalence of minor heart inflammation induced by PRV (when it occurred) in these experiments was demonstrated to be inconsequential to normal heart and respiratory functioning (Zhang et al., 2019). However, a correlation of PRV to moderate to severe heart inflammation has been proposed in a field environment based on a longitudinal study of one farm site with a high transient presence of moderate to severe cardiopathy (Di Cicco et al., 2017). The visualization of PRV in diseased hearts in this study in conjunction with activation of host cellular antiviral response pathways strongly suggested that PRV was contributing to the severity of cardiopathy observed. Thus, taken together, these studies suggest that PRV has the potential to exacerbate instances of severe cardiopathy in net-pen farmed Atlantic Salmon in British Columbia and may be a contributing etiological factor in establishing at least some instances of this relatively rare disease.

## PACIFIC SALMON

Two PRV experimental challenge studies have been published exploring the disease causing potential of PRV-1 to Sockeye Salmon; both failed to identify an association with PRV and disease (Garver et al., 2016a; Polinski et al., 2016). A third study has also recently been completed with Sockeye Salmon that failed to generate anemia or notable pathology in heart, kidney, or liver tissues of infected fish; and, in the same manner as assessing physiological impacts of PRV on Atlantic Salmon (Zhang et al., 2019), demonstrated these infections to be inconsequential to Sockeye physiological respiratory functioning (Polinski et al., manuscript in preparation). For adult Chilko or Shuswap Sockeye Salmon returning to the Fraser River, it was identified that the presence of PRV on or in the gills of fish migrating through Discovery or Juan De Fuca sea channels had no significant effect on the likelihood that they would reach their spawning grounds (Miller et al., 2014). Coho Salmon challenged with PRV harvested from infected farmed Atlantic Salmon in British Columbia also failed to acquire notable pathology or anemia despite attaining substantial PRV blood infections (Winton et al., manuscript in preparation). Lastly, experiments attempting to passage jaundice syndrome in Chinook Salmon in association with PRV failed to passage the disease despite successfully passing PRV (Garver et al., 2016b). Nevertheless, similar to field observation of PRV contributing to severe cardiopathy in Atlantic Salmon, PRV has been visualized in diseased tissues of farmed Chinook Salmon experiencing Jaundice Syndrome (Di Cicco et al., 2018) which would suggest that PRV is capable of contributing to jaundice/anemia in captive fish and may be part of a more complex aetiology for establishing this disease state in Chinook Salmon. However, this assumption cannot be extrapolated to wild Chinook Salmon or other Pacific Salmon because captive and wild environments can cause different pathophysiology and susceptibilities.

## DISEASE PREVENTION

In British Columbia, there has been no data to suggest PRV adversely affects aquaculture production of salmon. In Norway, however, HSMI is considered one of the most significant infectious diseases affecting Atlantic Salmon aquaculture (Hjeltnes B et al., 2017; Marine Harvest, 2017) and a number of strategies are being explored to mitigate this disease. Two experimental vaccination studies have been conducted; one using a formalin killed PRV vaccine (Wessel et al., 2018b) and one using a DNA vaccine expressing the non-structural proteins of PRV (Haatveit et al., 2018). Both demonstrated moderate protection against HSMI, although neither were protective against PRV infection. In addition to vaccination, work towards establishing a "HSMI-resistant" Atlantic Salmon strain has been undertaken (AquaGen, 2017), although similar to the vaccines mentioned above, these fish do not appear to be refractory to

PRV but rather only HSMI (Emilsen et al., 2017). Furthermore, use of specific feed formulations, similar to the other treatments, has shown promise at reducing the effects of HSMI primarily through dietary immunomodulation but is not successful at eliminating PRV infections (Grammes et al., 2012; Martinez-Rubio et al., 2012).

## SUMMARY

There has been a great deal of knowledge gained regarding the virology and ecology of PRV following its discovery nine years ago; and much of that knowledge has direct or indirect relevance for assisting in the assessment of risk to Fraser River Sockeye Salmon posed by the occurrence of PRV on Atlantic Salmon farms. The most critical research findings that can aid this risk assessment are summarized here:

- PRV is ubiquitous and highly prevalent in net-pen farmed salmon of British Columbia; it is also widely distributed in wild Pacific salmon but with less detection prevalence and more species/stock-specific variation.
- Farmed and wild salmon of British Columbia appear most likely to become infected with PRV as adults in saltwater although freshwater infections of fry/parr can and have occurred.
- Experimental infections with PRV in British Columbia generate high-load blood infections in both juvenile Atlantic and Pacific salmon but disease specific to PRV infection was not observed following experimental infection in any species challenged (Sockeye, Chinook, Coho, Pink and Atlantic).
- PRV is of lower virulence to Atlantic Salmon in British Columbia relative to PRV infections of Atlantic Salmon in Norway; both host and virus specific factors are likely involved in this altered virulence.
- Infections of PRV in Atlantic and Sockeye salmon of British Columbia have been demonstrated as inconsequential to respiratory function in the absence of moderate to severe pathology.
- Systemic PRV load is not indicative of whether a salmon will or will not develop a notable disease.
- PRV may contribute to or be a possible etiological component of severe heart inflammation in farmed Atlantic Salmon or jaundice/anemia syndrome in farmed Chinook Salmon in British Columbia; both conditions appear rare and likely have complex etiologies and have not been reported in wild Pacific salmon.
- There is currently no evidence to suggest that PRV causes disease in Sockeye Salmon, which appear less susceptible to infection relative to Atlantic Salmon in British Columbia.

Despite substantial gains in our understanding about PRV, there are also knowledge gaps concerning PRV virology and ecology that leave critical uncertainties. The environmental source(s) and transmission potential of PRV in ocean environments are unknown. Specifically, there is no current data on environmental shedding (quantity or duration) or the minimum exposure load (quantity or duration) to establish an infection in any salmon species. There is also a current lack of understanding for why PRV can show higher virulence in some instances compared to others. Lastly, in the instances where PRV has been linked to disease in farmed salmon, it is as yet unclear as to whether all host, environment, and viral specific factors of these diseases can manifest in the natural environment in British Columbia.

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**From:** DiCicco, Emiliano  
**Sent:** May-08-19 2:37 AM  
**To:** Miller-Saunders, Kristi  
**Subject:** RE: Summary of the PRV presentations

Yep

---

**From:** Miller-Saunders, Kristi  
**Sent:** May 7, 2019 9:27 PM  
**To:** DiCicco, Emiliano  
**Subject:** FW: Summary of the PRV presentations

We should discuss this tomorrow.

---

**From:** [REDACTED]  
**Sent:** May 7, 2019 9:17 PM  
**To:** [REDACTED] Miller-Saunders, Kristi  
**Subject:** Fwd: Summary of the PRV presentations

Hi [REDACTED]/Kristi

Please see email below from a member of the ENGO community concerning what is being presented on PRV by DFO at the Aquaculture Association of Canada conference being held in Victoria.

As I recall, none of the following "findings" or data related thereto were presented to us during the CSAS PRV risk assessment review. Please correct me if I am wrong

- the etiology and genetics component as described;
- the pathway of transfer of the virus (from NB To BC);
- the fact that PRV is found in the water around farms prior to fish entry and that all near farm water samples test(ed) positive for PRV;
- the fact that 350 species have been tested for the virus to try to find a reservoir;
- the fact naive fish (Atlantic's) in the lab never picked up the virus?(As I recall they always did ... in every lab test) ...;
- the fact that benthic samples were being taken to look for presence and prevalence of the virus;
- the fact that all hatcheries tested were free of PRV;
- and that the logical conclusion to be derived from all of this is that farmed salmon are contracting PRV from infected wild salmon.

And I also find it difficult to believe that they have discovered all this new information and published it in just three months?? (The CSAS review was at the end of January).

s.19(1)

Did I actually miss hearing about all of this during the review?

On May 7, 2019, at 3:52 PM, [REDACTED] wrote:

Hi, all—

You need to read this to believe it. Apologies for the length. Summary of the PRV presentations Day 2 of AAC Conference:

New publications by DFO on PRV completely reverse previous understandings. I had the opportunity to discuss the presentations with Andrew Bateman and Emiliano DiCiccio following; this note is a combination of the DFO evidence and their commentary.


- hatcheries are now free of PRV (E. DiCiccio says they're checking this right now; work has been done, but no firm conclusion)
  - PRV (BC) is genetically distinct from the Norwegian version; although no doubt descended from it, the divergence occurred in about 1715; it came first to New Brunswick and from there to BC in about 1950-1960 (except they only sequenced S1 segment according to DiCiccio and too few samples to draw any conclusions (per Bateman) and the confidence intervals span centuries) With all that uncertainty, which was not presented, the mere fact that they're putting this work forward speaks volumes about the extent of deception/misdirection to which they're prepared to descend.
  - although the time scales don't quite match, they suggest that the attempts to plant Atlantics on the west coast were the vector for transmission of the virus to BC (1905-1935)—OR possibly the importation of +/- 35 million imported salmon eggs, 1985-2009
  - Atlantics are now going into the ocean PRV-free, but 100% of them develop PRV within 100-200 days
  - PRV levels are easily measured in the water; all of their near-farm samples are positive @ at least 10,000 copies per litre. There's lots of the stuff out there. Some water samples were positive even before the fish were testing positive for PRV, meaning it didn't come from the fish shedding the virus. (We have no info on how long PRV can survive in the ocean. Some viruses die within ½ hour; some take days. DiCiccio: some human viruses can live for a year without a host. We just don't know.)
  - they checked 350 species for a reservoir species and they were all clean
  - they have benthic samples, but haven't yet checked them out as a potential reservoir.
  - a test done in both tanks and the ocean at Departure Bay with naïve Atlantics: they never picked up PRV.
  - the logical conclusion is that the wild fish carry PRV (and have done since ~1950) and they're infecting the farmed salmon (since the prevalence in the wild is only 3%, must be pretty damned infectious –DiCiccio)
- The Minister's upcoming PRV announcement was discussed. [REDACTED] in particular, was derisive about the idea that 'precaution' might suggest it's a good idea to keep PRV infected smolts out of the ocean. Marty got up to suggest it would be safer to infect smolts and wait until they've passed the shedding stage before putting them in the water.

Polinski said we really can't say when they've stopped shedding, so that won't work; but since all Atlantics are going to get PRV once in the water, there seems little point in putting 'clean' smolts in.

Oddly, they say that even in the DI, where fallowing occurs in tandem for all farms, the fish are still getting PRV within 100-200 days.

An experiment conducted in both tanks and the ocean at Departure Bay put naïve Atlantics in the water, unfiltered in the case of the tanks. These salmon did not contract PRV. I guess no wild salmon visit Departure Bay.

There's much more new intel from this conference, but I thought it important to get this to you in writing quickly, as it will be important to bear in mind what the Minister will have heard when he announces his new PRV policy.




Living Oceans Society  
Suite 7, 650 Clyde Avenue  
West Vancouver BC V7T 1E2

Ph: 604-696-5044

Fax: 604-696-5045

Cell: 

[www.livingoceans.org](http://www.livingoceans.org)<http://www.livingoceans.org/>



<image001.png>

s.19(1)



## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** May-08-19 6:22 AM  
**To:** Miller-Saunders, Kristi  
**Subject:** Fwd: Summary of the PRV presentations  
**Attachments:** image001.png

[REDACTED] I have meetings through mid-afternoon today but could call you then if you are available?

Begin forwarded message:

**From:** [REDACTED]  
**Date:** May 7, 2019 at 10:17:44 PM PDT  
**To:** "[REDACTED]"  
**Cc:** [REDACTED]

**Subject:** RE: Summary of the PRV presentations

I've asked [REDACTED] to check it out with [REDACTED] Kristi to see what they think of a CSAS being done without any of this intel.

[REDACTED]  
Living Oceans Society  
Suite 7, 650 Clyde Avenue  
West Vancouver BC V7T 1E2  
Ph: 604-696-5044  
Fax: 604-696-5045  
Cell: [REDACTED]  
[www.livingoceans.org](http://www.livingoceans.org)<<http://www.livingoceans.org/>>

Skype: [REDACTED]  
[LOS logo]

s.19(1)

**From:** [REDACTED]  
**Sent:** Tuesday, May 7, 2019 7:49 PM

To:

Cc:

Subject: Re: Summary of the PRV presentations

All,

I really didn't think they could go lower...we need to expose this. It's outrageous.

From:

Date: Tuesday, May 7, 2019 at 6:04 PM

To:

Cc:

Subject: Re: Summary of the PRV presentations

OMG ...

Much of this was "NOT" made known during the PRV CSAS risk assessment. To wit; the etiology and genetics component ... the paths of transfer (NB -BC), the fact that PRV is found in the water around farms prior to fish entry, the checking of 350 fish species for the virus, all near farm samples are positive for PRV?? (We have data that suggest otherwise), the fact naive fish (Atlantic's) never picked up the virus?(they always did ... in every lab test) ... and they discovered all this information and published it in just three months?? (The CSAS review was at the end of January).

Wow! ... just Wow!

Glad I am not there.

But surprised people in our group are. I asked a while back if anyone was attending and the answer was "No",

But glad you are.

On May 7, 2019, at 3:52 PM, [REDACTED] wrote:  
Hi, all—

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- ? a test done in both tanks and the ocean at Departure Bay with naïve Atlantics: they never picked up PRV.
- ? the logical conclusion is that the wild fish carry PRV (and have done since ~1950) and they're infecting the farmed salmon (since the prevalence in the wild is only 3%, must be pretty damned infectious –DiCiccio)  
The Minister's upcoming PRV announcement was discussed. [REDACTED] in particular, was derisive about the idea that 'precaution' might suggest it's a good idea to keep PRV infected smolts out of the ocean. Marty got up to suggest it would be safer to infect smolts and wait until they've passed the shedding stage before putting them in the water. Polinski said we really can't say when they've stopped shedding, so that won't work; but since all Atlantics are going to get PRV once in the water, there seems little point in putting 'clean' smolts in.


s.19(1)


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An experiment conducted in both tanks and the ocean at Departure Bay put naïve Atlantics in the water, unfiltered in the case of the tanks. These salmon did not contract PRV. I guess no wild salmon visit Departure Bay.

There's much more new intel from this conference, but I thought it important to get this to you in writing quickly, as it will be important to bear in mind what the Minister will have heard when he announces his new PRV policy.



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<image001.png>

s.19(1)

## Miller-Saunders, Kristi

**From:** Higgins, Mark  
**Sent:** May-09-19 9:57 AM  
**To:** MacDougall, Lesley; Miller-Saunders, Kristi; Holmes, John; Kennedy, Eddy; Neville, Chrys; Beacham, Terry; Withler, Ruth; MacWilliams, Christine; Garver, Kyle; Jones, Simon  
**Subject:** RE: your input requested: DFO response to court decision - comments due by MONDAY  
**Attachments:** [REDACTED]

[REDACTED] Mark.

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** May-09-19 7:56 AM  
**To:** Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Holmes, John <John.Holmes@dfo-mpo.gc.ca>; Kennedy, Eddy <Eddy.Kennedy@dfo-mpo.gc.ca>; Neville, Chrys <Chrys.Neville@dfo-mpo.gc.ca>; Beacham, Terry <Terry.Beacham@dfo-mpo.gc.ca>; Withler, Ruth <Ruth.Withler@dfo-mpo.gc.ca>; MacWilliams, Christine <Christine.MacWilliams@dfo-mpo.gc.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Jones, Simon <Simon.Jones@dfo-mpo.gc.ca>  
**Subject:** your input requested: DFO response to court decision - comments due by MONDAY

Hi all – PLEASE SHARE THIS WITH others in your groups if I’ve missed someone who would like to weigh in.

Please note below, [REDACTED]  
[REDACTED]  
[REDACTED]

We have until Monday to provide comments. I’ll schedule a meeting for Monday morning for us to quickly review / consolidate comments from our science group to send back. In the meantime, please review the documents and share your comments using track changes, with THIS group for now (i.e. let’s consolidate before we send input back to NHQ).

Thanks in advance;  
Lesley

s.21(1)(a)

s.21(1)(b)

s.23

**Subject:** [REDACTED]

Hi all,

First, a big thank you to everyone for all the great comments [REDACTED] John Martell and I have done our utmost to address as much of your feedback as possible, and I think we have a much more mature product now. Not perfect, but getting there!

Attached is the next version of the doc—all nice, shiny, clean, and formatted—ready for your red pens to carve it up again! Hopefully (hopefully), the carnage will be greatly reduced on this version. ;-)

If it is possible, it would be great if we could get consolidated edits from each group to keep things manageable. Brenda and Phil, any suggestions you have for plainer language are greatly appreciated! Matt, [REDACTED]  
[REDACTED]

The due date for comments on this version is COB Monday, May 13. Earlier is always appreciated! ☺

Note: [REDACTED], with CFIA yesterday, in advance of a  
DG-level meeting with them this Friday, May 10.

Let me know if you have any questions!  
Cheers,  
Roderick.

s.21(1)(a)  
s.21(1)(b)  
s.23

No further information has been removed or severed from this page

**Pages 540 to / à 552**  
**are withheld pursuant to section**  
**sont retenues en vertu de l'article**

**23**

**of the Access to Information Act**  
**de la Loi sur l'accès à l'information**

## Miller-Saunders, Kristi

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**Subject:** FW: 56 Framework Documents & Chinook Analysis Review  
**Location:** 1-877-413-4788 Code [REDACTED]  
  
**Start:** Fri 10/05/2019 1:30 PM  
**End:** Fri 10/05/2019 2:30 PM  
**Show Time As:** Tentative  
  
**Recurrence:** (none)  
  
**Meeting Status:** Not yet responded  
  
**Organizer:** Paylor, Adrienne

Hello all – this call is an update on the PRV documents that are moving forward – information, update on progress, questions, discuss concerns and next steps.

Please join me in my office if you are available; if you're not available I'll send out a short re-cap afterward.

Thanks  
Lesley

-----Original Appointment-----

**From:** Paylor, Adrienne  
**Sent:** May-08-19 11:18 AM  
**To:** Paylor, Adrienne; Parsons, Jay; Burgetz, Ingrid; MacDougall, Lesley; Higgins, Mark; McCorquodale, Brenda; Price, Derek  
**Subject:** 56 Framework Documents & Chinook Analysis Review  
**When:** May-10-19 1:30 PM-2:30 PM (UTC-08:00) Pacific Time (US & Canada).  
**Where:** 1-877-413-4788 Code [REDACTED]

Hi All,

Brenda asked me to set up a call to discuss all the moving PRV pieces as a group (Guidance doc, Practitioners Guide, Aggregates paper, consultation package? & Chinook Analysis etc.).

This is the only time I could find according to everyone's calendars but might be a bit late in the day/week for some of these pieces? I will start with this time proposal and we can work it from there (Jay you look fully booked any other time). Please let me know if there are others (John Holmes? AMD NCR?) you may want to include.

Thanks,  
Adrienne

s.16(2)(c)



## Miller-Saunders, Kristi

---

**From:** Holmes, John  
**Sent:** May-10-19 12:37 PM  
**To:** MacDougall, Lesley; Higgins, Mark; Miller-Saunders, Kristi; Kennedy, Eddy; Neville, Chrys; Beacham, Terry; Withler, Ruth; MacWilliams, Christine; Garver, Kyle; Jones, Simon  
**Subject:** RE: your input requested: DFO response to court decision - comments due by MONDAY  
**Attachments:** [REDACTED]

My comments in the attached documents.

—  
John

(250) 756-7145  
[John.Holmes@dfo-mpo.gc.ca](mailto:John.Holmes@dfo-mpo.gc.ca)

---

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** May-09-19 7:56 AM  
**To:** Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Holmes, John <John.Holmes@dfo-mpo.gc.ca>; Kennedy, Eddy <Eddy.Kennedy@dfo-mpo.gc.ca>; Neville, Chrys <Chrys.Neville@dfo-mpo.gc.ca>; Beacham, Terry <Terry.Beacham@dfo-mpo.gc.ca>; Withler, Ruth <Ruth.Withler@dfo-mpo.gc.ca>; MacWilliams, Christine <Christine.MacWilliams@dfo-mpo.gc.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Jones, Simon <Simon.Jones@dfo-mpo.gc.ca>  
**Subject:** your input requested: DFO response to court decision - comments due by MONDAY

Hi all – PLEASE SHARE THIS WITH others in your groups if I’ve missed someone who would like to weigh in.

Please note below, [REDACTED]

We have until Monday to provide comments. I’ll schedule a meeting for Monday morning for us to quickly review / consolidate comments from our science group to send back. In the meantime, please review the documents and share your comments using track changes, with THIS group for now (i.e. let’s consolidate before we send input back to NHQ).

Thanks in advance;  
Lesley

**Subject:** [REDACTED] s.21(1)(a)  
s.21(1)(b)  
s.23

Hi all,

First, a big thank you to everyone for all the great comments on last week’s version of the “Guidance Document”. John Martell and I have done our utmost to address as much of your feedback as possible, and I think we have a much more mature product now. Not perfect, but getting there!

Attached is the next version of the doc—all nice, shiny, clean, and formatted—ready for your red pens to carve it up again! Hopefully (hopefully), the carnage will be greatly reduced on this version. ;-)  
If it is possible, it would be great if we could get consolidated edits from each group to keep things manageable. Brenda and Phil, any suggestions you have for plainer language are greatly appreciated! Matt, [REDACTED]  
[REDACTED]

The due date for comments on this version is COB Monday, May 13. Earlier is always appreciated! ☺

Note: this version of the GD was shared, along with the latest practitioner's guide, with CFIA yesterday, in advance of a DG-level meeting with them this Friday, May 10.

Let me know if you have any questions!

Cheers,  
Roderick.

s.21(1)(a)

s.21(1)(b)

s.23

**Pages 556 to / à 584**  
**are withheld pursuant to section**  
**sont retenues en vertu de l'article**

**23**

**of the Access to Information Act**  
**de la Loi sur l'accès à l'information**

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** May-13-19 3:40 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re: 2019 - BCCAHS - 20190506

OK, thanks this is as expected!

[REDACTED]

> On May 13, 2019, at 3:15 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>

> It is important that they use the procedure that they intend to use moving forward, whether that is their original extraction protocol or the Trizol protocol we sent to them, what we need to know is that they get comparable data starting their processing at different steps along the technical pathway. I should be clear to you, [REDACTED] a proportion of the samples provided are consistent between each step (i.e. same samples each step) and others are "random"--i.e. samples whereby we know the result but they may have not gotten the same sample for each step. [REDACTED] In this way, they should not expect the patterns to necessarily match between sets of 20. And most certainly the orders of the samples for each step do not match. [REDACTED]

>

> Kristi Miller-Saunders, PhD  
> Head, Molecular Genetics  
> Pacific Biological Station  
> 3190 Hammond Bay Rd  
> Nanaimo BC V9T 6N7  
> 250-756-7155  
> Kristi.Saunders@dfo-mpo.gc.ca

s.19(1)

>

s.21(1)(b)

>

> -----Original Message-----

> From: [REDACTED]  
> Sent: May-13-19 1:50 PM  
> To: Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
> Subject: Fwd: 2019 - BCCAHS - 20190506

>

> Could you please read this over ... I think he is proposing what we asked??!! We want them to do the initial extraction using their usual procedure. But after extraction, do they need to fully process the sample in order to fully test for PRV load?

>

> [REDACTED]

>

> Begin forwarded message:

>

> From: [REDACTED]  
> Date: May 13, 2019 at 1:41:32 PM PDT  
> To: [REDACTED]

> Subject: RE: 2019 - BCCAHS - 20190506

>

> [REDACTED]

>

> In consultation with members of my BOD and in the interest of getting things going, let me propose something.

>

> The 100 samples are actually 5 sets of the same 20 tissue samples that are processed from start to finish using the MagMax protocol. That is, the 20 samples were processed and subsamples of the five step protocol were removed at four milestones in the protocol.

>

> The objective of the exercise was to see if we got the same results at each step of the protocol that PSF did. And if the results differed, we would know where the train went off the rails and do something about it.

>

> We don't have the PSF/PBS MagMax protocol. We've used the Trizol manual method supplied by PSF to process the Broughton samples so far.

>

> To test the steps of the MagMax protocol using our Trizol manual method would not be a head-to-head comparison. And, we would not know if there was an impact on the integrity of the step-wise products. It would generate more questions.

>

> So how about this: We can run the 20 'fresh' (unextracted) samples by the Trizol manual method we used on the Broughton samples so far.

>

> If the results are way out, say greater than 3Ct values (10x difference) in the below 30 range for 80% of the samples, then we dig deeper into the issue.

>

> Under 3Ct values in 80% of the samples and there is no need to go further, the methods are acceptable.

>

> As PRV L1 is the most prevalent pathogen concern, I suggest we look for that target.

>

> This proposition would save time and money and be informative so we can resume testing the samples to everyone's satisfaction. In reality, we're comparing apples to apples using different methods by doing the fresh samples first. (Which was the plan anyway.)

>

> If this sounds acceptable to you, we'll arrange for the samples to be picked up and we'll get going.

>

> Please let me know,

>

> [REDACTED]

>

>

s.19(1)

> From: [REDACTED]

> Sent: May 10, 2019 3:00 PM

> To: [REDACTED]

> Subject: RE: 2019 - BCCAHS - 20190506

>

> [REDACTED]

>

> Any updates from your end?

>

> With thanks,

>

> [REDACTED]  
>  
> From: [REDACTED]  
> Sent: May 8, 2019 8:20 AM  
> To: [REDACTED]  
> Subject: RE: 2019 - BCCAHS - 20190506  
>  
> Thanks, [REDACTED]  
>  
> [REDACTED]  
>  
> From: [REDACTED]  
> Sent: May 8, 2019 8:20 AM  
> To: [REDACTED]  
> Subject: RE: 2019 - BCCAHS - 20190506  
>  
> Hi [REDACTED]  
>  
> We're considering this at the Board level and we will have a reply for you soon.  
>  
> Thank you for your patience.  
>  
> [REDACTED]  
>  
> From: [REDACTED]  
> Sent: May 7, 2019 8:19 AM  
> To: [REDACTED]  
> Subject: RE: 2019 - BCCAHS - 20190506  
>  
> Hi [REDACTED]  
>  
> Just following up on my earlier email.  
>  
> [REDACTED]  
>  
> From: [REDACTED]  
> Sent: May 6, 2019 9:39 AM  
> To: [REDACTED]  
> Subject: 2019 - BCCAHS - 20190506  
>  
> Hi [REDACTED]  
>  
> Would you have a few minutes to discuss the draft agreement attached. Once this is settled the samples should be ready to be picked up.  
>  
> Looking forward to discussing this with you.  
>  
> [REDACTED]

s.19(1)

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** May-14-19 3:00 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Fwd: PRV impact on juvenile Chinook  
**Attachments:** Chinook infectious migrating Wang.pdf; ATT00001.htm

Since you signed this thesis I am assuming you were well aware of it. Was this supported at all by PSF ... just surprised we knew nothing about it.

[REDACTED]

Begin forwarded message:

**From:** [REDACTED]  
**To:** [REDACTED]

**Subject:** PRV impact on juvenile Chinook

Hello

I came across this Masters Thesis, that I had not seen before. He not only found PRV in juvenile Chinook, but also found that it appears that the virus is harming the fish. The fish appeared to be in the early stages of jaundice, however, he didn't find high loads, or advanced disease and wonders if that may suggest that the fish died before reaching that stage.

He reports a high percentage of the infected Chinook in regions identified in Morton et al 2017, where we report higher prevalence of the virus in other species in association with high-density salmon farms.

He also found higher prevalence of PRV in hatchery Chinook than wild...

I have highlighted key methods and points.

It makes me wish I had access to Quatsino Sound for sea lice and other research as young Chinook in that region are particularly infected.

This work should be considered by Wilkinson in his decision whether to screen for PRV, if any are meeting with him

THE PHYSIOLOGICAL ASSOCIATIONS BETWEEN INFECTIOUS AGENTS AND  
MIGRATING JUVENILE CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*)

by

Yuwei Wang

B.Sc., Xiamen University, 2016

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF

MASTER OF SCIENCE

in

The Faculty Of Graduate And Postdoctoral Studies  
(Forestry)

THE UNIVERSITY OF BRITISH COLUMBIA  
(Vancouver)

December, 2018

© Yuwei Wang, 2018



**Pages 590 to / à 702  
are withheld pursuant to section  
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**68(a)**

**of the Access to Information Act  
de la Loi sur l'accès à l'information**

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** May-14-19 3:26 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** RE: No comment?

Hi Kristi:

Thank you for sharing your thoughts on this.

I have a couple of questions.

1) When was it determined that all of the hatcheries were free of PRV? The data presented in the CSAS working papers showed a decline in infectivity in hatcheries over time but it was never stated that they were free of PRV.

2) These 350 marine fish species .... that's a lot of different species and I'm sure it took a while to capture and analyze each and every one for presence/absence of PRV. When was this work undertaken? I find it hard to believe that anyone could capture so many different species and sample them for the virus in just three short months.

3) you mention in your comments that the water sample data was part of the Polinsky talk and that means they did have this data at the time of the CSAS. But correct me if I am wrong ... as I recall, they did not present these data during the CSAS. In fact, I recall them saying just the opposite ... they said there were no data available to determine whether PRV could be detected outside of farms in any quantity. IMO opinion had they shared these data, things might have turned out differently because the insinuation from these data is in the water near fish farms, PRV viral load is high.

Thanks

-----Original Message-----

From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]  
Sent: Monday, May 13, 2019 9:56 AM  
To: [REDACTED]  
Subject: RE: No comment?

Here are my comments on what has been discussed. I was not at the meeting and thus did not see these talks, but I did discuss with Emiliano. I have no idea if these are new data or data that was available prior to the CSAS.

Kristi Miller-Saunders, PhD  
Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

s.19(1)

-----Original Message-----

From: [REDACTED]

Sent: May-10-19 12:16 PM

To: [REDACTED]

Cc: Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>

Subject: Re: No comment?

With apologies where's been very little time to direct to this and Kristi and I have not even discussed yet. I will try to discuss

[REDACTED]

> On May 10, 2019, at 11:51 AM, [REDACTED] wrote:

>

> Hey [REDACTED]/Kristi

>

> Just curious as to your thoughts on the latest PRV info that I forwarded.

>

> I will keep your responses in confidence, but before I or anyone else speak to this (if we do ...) knowing we are not being flippant or mid-informed in our response would be good.

>

> I am thinking that I would like to raise some of these issues with Jay Parsons in regards to the CSAS review (if there are valid concerns .. and I think there are) ... and that would be me asking him how this should play out.

>

> It may very well be that DFO has gone all out and gathered all of this new info post-CSAS. I don't know.

>

> That's why I' reaching out.

>

[REDACTED]

s.19(1)

## Miller-Saunders, Kristi

---

**From:** Miller-Saunders, Kristi  
**Sent:** May-14-19 9:20 PM  
**To:** MacDougall, Lesley; Higgins, Mark  
**Subject:** RE: follow up from ministerial briefing  
**Attachments:** Wang MSc thesis - Chinook infectious agents - PRV.pdf

I am not sure what "new" conclusions [REDACTED] has made? Is this something recent? How are we supposed to know her sources if we (or I) don't know what her conclusions are?

I did receive an email from [REDACTED] asking about a MSc thesis that came out of my program this past year that Alex recently found. I have enclosed it herein, but I did provide this to those preparing the PRV CSAS, and it was entirely ignored. It offers the first evidence that the lesions consistent with jaundice/anemia, and their relationship with PRV, can also be observed in wild salmon, and that transcriptionally, PRV is associated with the most powerful response of all of the microbes carried by wild salmon.

Kristi

---

**From:** MacDougall, Lesley  
**Sent:** May 14, 2019 5:15 PM  
**To:** Higgins, Mark; Miller-Saunders, Kristi  
**Subject:** follow up from ministerial briefing

Hi both – the Minister was briefed today on aquaculture related issues (I was not part of the briefing), and as a result we have some homework!  
We have been asked to:

Work with AMD to prepare a briefing on sea lice: basic science, background, treatment, current situation.  
I'll be away from the office tomorrow – the briefing is apparently next week, so if we can get a draft to share with AMD Thursday am that would be great. – they're looking for a pp deck

We've also been asked if we know what sources of science [REDACTED] may be using to reach her conclusions. (I believe the Minister is planning to meet [REDACTED] next week and has asked [REDACTED] to participate too – hence the briefing request).

I'll look for a bit of time Thursday afternoon where we can get together and look through what we've got and what might be useful for the Minister. At this point the ask is a bit nebulous except for the sea lice briefing for next week....

Thanks;  
Lesley

s.19(1)

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** May-14-19 9:30 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re: PRV impact on juvenile Chinook

This not accurate. Minister has agreed to meet [REDACTED] at her request. I am not aware of her intent but likely sea lice in BA now at about 90% incidence. Minister asked if I could also attend but I am away. That's all I know.

[REDACTED]

> On May 14, 2019, at 9:09 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>

> I got a message from Lesley about providing briefing materials to the minister "We've also been asked if we know what sources of science [REDACTED] may be using to reach her conclusions. (I believe the Minister is planning to meet [REDACTED] next week and has asked [REDACTED] to participate too – hence the briefing request)". Do you think this request pertains to the Wang study? Not sure what "her conclusions" are? Is there something new I don't know about?

>

> From [REDACTED]  
> Sent: May 14, 2019 3:58 PM  
> To: Miller-Saunders, Kristi  
> Subject: Re: PRV impact on juvenile Chinook

>

> It is definitely pertinent and is another independent observation in the wild. I don't recall the other Chinook issue but again this is additional evidence that PRV does have associated risks with Chinook and Coho. Is the latter one documented?

>

> [REDACTED]

>

>> On May 14, 2019, at 3:53 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>>

>> Yes we are, and we have in situ to go with it, but if DFO dismissed our last paper outright where we show PRV in situ in the developing lesions of farmed Chinook salmon and provide a mechanistic understanding of how this disease likely manifests through lysis of RBCs and hemoglobin overload, then this data showing the same pattern in wild Chinook salmon will not likely sway them. And I did send them the thesis. I did not like some of the way she presented the work--very poor job at describing the histo, and unbalanced job between the agents, but it is pertinent.

>>

>> Not sure if Emiliano or I told you about the issues with HSML-like disease with PRV involvement in New Zealand Chinook? He has been asked by the Chileans to help the New Zealander's figure this out. Don't know the details. This would also play in our favour, regardless of what strain of PRV it is, showing a similar disease in Chinook elsewhere in the world. But I don't know if the manifestation is similar to Atlantic salmon or Coho.

>>

>> Kristi Miller-Saunders, PhD  
>> Head, Molecular Genetics  
>> Pacific Biological Station  
>> 3190 Hammond Bay Rd  
>> Nanaimo BC V9T 6N7  
>> 250-756-7155

s.19(1)

>> Kristi.Saunders@dfo-mpo.gc.ca

>>

>>

>> -----Original Message-----

>> From: [REDACTED]

>> Sent: May-14-19 3:48 PM

>> To: Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>

>> Subject: Re: PRV impact on juvenile Chinook

>>

>> Thanks, my apologies as I have no recall of this! Seems very important right now given the added push from DFO (recent presentations). I wonder if it could be worth summarizing and notifying DFO?? You and Emiliano are confident in the analysis?

>>

>>

>> [REDACTED]

>>

>>> On May 14, 2019, at 3:42 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>>>

>>> Yovella (a she) was a graduate student with Scott and myself and I have discussed this work with you, and have mentioned it in various reports and at our annual review. I also provided it to Jay Parsons for the CSAS-- [REDACTED]

[REDACTED]

>>>

>>> This is the first demonstration that PRV may be causing disease in wild Chinook salmon, and Emiliano even saw the advanced necrotic kidney tubules in some fish. This was one of the reasons we went back to Quatsino this year, to try to get more fish, but unfortunately few were PRV positive this year. Other than the thesis, she has not published this work unfortunately. It needs some work to get it paper ready. All of the technical work was done in my lab, histo by Emiliano, and she was handed the data to work up.

>>>

>>> Kristi Miller-Saunders, PhD

>>> Head, Molecular Genetics

>>> Pacific Biological Station

>>> 3190 Hammond Bay Rd

>>> Nanaimo BC V9T 6N7

>>> 250-756-7155

>>> Kristi.Saunders@dfo-mpo.gc.ca

>>>

>>>

>>> -----Original Message-----

>>> From: [REDACTED]

>>> Sent: May-14-19 3:00 PM

>>> To: Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>

>>> Subject: Fwd: PRV impact on juvenile Chinook

>>>

>>> Since you signed this thesis I am assuming you were well aware of it. Was this supported at all by PSF ... just surprised we knew nothing about it.

s.19(1)

>>>

s.21(1)(b)

>>> [REDACTED]

>>>

>>> Begin forwarded message:

>>>

>>> From: [REDACTED]

>>> To: "

>>> Subject: PRV impact on juvenile Chinook

>>>

>>> Hello

>>>

>>> I came across this Masters Thesis, that I had not seen before. He not only found PRV in juvenile Chinook, but also found that it appears that the virus is harming the fish. The fish appeared to be in the early stages of jaundice, however, he didn't find high loads, or advanced disease and wonders if that may suggest that the fish died before reaching that stage.

>>>

>>> He reports a high percentage of the infected Chinook in regions identified in Morton et al 2017, where we report higher prevalence of the virus in other species in association with high-density salmon farms.

>>>

>>> He also found higher prevalence of PRV in hatchery Chinook than wild...

>>>

>>> I have highlighted key methods and points.

>>>

>>> It makes me wish I had access to Quatsino Sound for sea lice and other research as young Chinook in that region are particularly infected.

>>>

>>> This work should be considered by Wilkinson in his decision whether to screen for PRV, if any are meeting with him

>>>

>>>

>>>

s.19(1)

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** May-16-19 3:33 PM  
**To:** Kaukinen, Karia; [REDACTED] Schulze, Angela; Li, Shaorong  
**Cc:** DiCicco, Emiliano; Miller-Saunders, Kristi; Ming, Tobi; [REDACTED]  
**Subject:** RE: Samples for BC CAHS Bench Validation

Hi Karia,  
Your assumption is correct, I will be there at approx. 10am –

[REDACTED]  
Thanks  
[REDACTED]

**From:** Kaukinen, Karia [mailto:Karia.Kaukinen@dfo-mpo.gc.ca]  
**Sent:** Thursday, May 16, 2019 3:27 PM  
**To:** [REDACTED] Schulze, Angela <Angela.Schulze@dfo-mpo.gc.ca>; Li, Shaorong <Shaorong.Li@dfo-mpo.gc.ca>  
**Cc:** DiCicco, Emiliano <Emiliano.DiCicco@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Ming, Tobi <Tobi.Ming@dfo-mpo.gc.ca>; [REDACTED]  
**Subject:** RE: Samples for BC CAHS Bench Validation

Hi,  
Since you did not include a time, I cannot confirm the exact person. However, between the hours of 5am and usually 6pm, there are people within our group in the lab. I am assuming that [REDACTED] will leave Campbell River in the morning arriving here by approximately 10am? I will notify the commissionaire that we are expecting him and to call up to one or more of us to ensure [REDACTED] turn around time is quick.

[REDACTED]  
Karia

---

**From:** [REDACTED]  
**Sent:** 16 May 2019 16:34  
**To:** Kaukinen, Karia; Schulze, Angela; Li, Shaorong  
**Cc:** DiCicco, Emiliano; Miller-Saunders, Kristi; Ming, Tobi; [REDACTED]  
**Subject:** RE: Samples for BC CAHS Bench Validation

Hello Karia,

Thank you for the quick reply. [REDACTED] will drive to Nanaimo on Tuesday (May 21<sup>st</sup>) to pick up the samples. He will have the cooler and dry ice for immediate return to CAHS.

Please confirm that someone will be available to meet [REDACTED] on Tuesday.

Regards,

s.19(1)

[REDACTED]  
**From:** Kaukinen, Karia <Karia.Kaukinen@dfo-mpo.gc.ca>  
**Sent:** May 16, 2019 12:17 PM  
**To:** [REDACTED]; Schulze, Angela <Angela.Schulze@dfo-mpo.gc.ca>; Li, Shaorong



<Shaorong.Li@dfo-mpo.gc.ca>

**Cc:** DiCicco, Emiliano <Emiliano.DiCicco@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Ming, Tobi <Tobi.Ming@dfo-mpo.gc.ca>

**Subject:** RE: Samples for BC CAHS Bench Validation

Hi,

The sample trays are all in a clearly labelled box ready to go. If you are going to have someone pick them up, they will need dry ice and cooler. Cooler large enough to accommodate the box measuring 8.5" wide, 6" tall and 5" deep and approximately 10lbs of dry ice (if returning immediately to CAHS), otherwise 25 lbs of dry ice for less direct transport.  
Karia

**From:** [REDACTED]

**Sent:** May-16-19 11:26 AM

**To:** Kaukinen, Karia <Karia.Kaukinen@dfo-mpo.gc.ca>; Schulze, Angela <Angela.Schulze@dfo-mpo.gc.ca>; Li, Shaorong <Shaorong.Li@dfo-mpo.gc.ca>

**Subject:** Samples for BC CAHS Bench Validation

Hello,

My name is [REDACTED] here at BC CAHS in Campbell River. I understand we are to coordinate the shipping of 20 samples to Campbell River for RT-qPCR screening for PRV.

Please let me know when the samples are ready for shipment or pickup, whichever is easier and more convenient for you.

Kind regards,

[REDACTED]

-----  
[REDACTED]

BC Centre for Aquatic Health Sciences  
Street Address: 871A Island Hwy, Campbell River, BC  
Mailing Address: PO Box 25070 Tyee, Campbell River, BC, Canada V9W 0B7  
ph: 250 286-6102 f: 250 286-6103  
email: [REDACTED]  
web: [www.cahs-bc.ca](http://www.cahs-bc.ca)

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**BC CAHS Celebrating 14 Years 2005 - 2019**

s.19(1)

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** May-16-19 3:57 PM  
**To:** [REDACTED]  
**Cc:** Miller-Saunders, Kristi  
**Subject:** Re: Just curious

I have zero knowledge of this review at this time.

[REDACTED]

> On May 16, 2019, at 9:51 AM, [REDACTED] wrote:

>

> Have either of you been invited to participate in the next CSAS pathogen review? (Tenacibaculum maritimum)?

>

> I was asked by Jay Parsons to be a part of the SC for that review. He said he wanted to keep the SC composition the same as for PRV.

>

> [REDACTED]

>

> [REDACTED]. I realize you were not on the SC for PRV but you were an invited participant in that review.

>

> Has that changed?

>

> [REDACTED]


s.19(1)

## Miller-Saunders, Kristi

---

**From:** Omid Nekouei <onekouei@upei.ca>  
**Sent:** May-21-19 2:46 PM  
**To:** Kaukinen, Karia; Ming, Tobi  
**Cc:** Miller-Saunders, Kristi  
**Subject:** A quick question

Hello Karia and Tobi,

 I am working on a PRV file and have a quick question if you do not mind to check for me.

What was the overall number of PRV-positive samples out of 655 juvenile Chinook tested in Strahan's PLOS-ONE paper? The total number of positive samples/655 is not mentioned in the paper. (I asked Strahan first, but he could not remember and referred me to you.)

Thank you very much in advance!!

Omid

s.19(1)

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** May-16-19 5:46 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re: Just curious

Thanks ... T. Maritimum is a nasty bug. Causing more health events on BC farms than any other from what I can see (mouthrot).

I noticed that a UBC Masters Student (Yuwei Wong?) recently published his thesis ... and it mentions finding T. Maritimum in wild chinook .. hopefully his data can be used to inform the review (proxy data seeing as it is not FR sockeye).

Question: How does one contact you outside of work the work space? Is there another way to reach you?

[REDACTED]

> On May 16, 2019, at 12:09 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>  
> Yes  
>  
> Kristi Miller-Saunders, PhD  
> Head, Molecular Genetics  
> Pacific Biological Station  
> 3190 Hammond Bay Rd  
> Nanaimo BC V9T 6N7  
> 250-756-7155  
> Kristi.Saunders@dfo-mpo.gc.ca s.19(1)

>  
>  
> -----Original Message-----

> From: [REDACTED]  
> Sent: May-16-19 11:21 AM  
> To: Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
> Subject: Re: Just curious

>  
> Did you accept?

[REDACTED]

>> On May 16, 2019, at 10:04 AM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>>  
>> I was asked. Was not told that the composition would be the same however.

>> \_\_\_\_\_  
>> From: [REDACTED]

>> Sent: May 16, 2019 9:50 AM

>> To: Miller-Saunders, Kristi; [REDACTED]

>> Subject: Just curious

>>

>> Have either of you been invited to participate in the next CSAS pathogen review? (Tenacibaculum maritimum)?

>>

>> I was asked by Jay Parsons to be a part of the SC for that review. He said he wanted to keep the SC composition the same as for PRV.

>>

>> [REDACTED]

>>

>> [REDACTED] I realize you were not on the SC for PRV but you were an invited participant in that review.

>>

>> Has that changed?

>>

> [REDACTED]  
> [REDACTED]  
> [REDACTED]

s.19(1)

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** May-22-19 10:51 AM  
**To:** Kaukinen, Karia; [REDACTED]; Schulze, Angela; Li, Shaorong  
**Cc:** DiCicco, Emiliano; Miller-Saunders, Kristi; Ming, Tobi; [REDACTED]  
**Subject:** RE: Samples for BC CAHS Bench Validation

Hi Karia,

Thank you for providing the samples yesterday –

Here is the inventory of the samples and plates that we have received:

- Plate 1a: 8 tubes with tissues labelled A to H and 8 tubes with tissues labelled A to H
- Plate 2b: 8 tubes with tissues labelled A to H and 8 tubes with tissues labelled A to H
- Plate 3: 16 wells with liquid (assuming aqueous layer)
- Plate 4: 16 wells with liquid (assuming Total RNA)
- Plate 5: 16 wells with liquid (assuming normalized RNA)
- Plate 6: 16 wells with liquid (assuming cDNA)
- Plate 7: 8 wells with liquid (assuming DNA)

Please could you confirm if this is correct?

On the spread-sheet, you mentioned 16 kidney/liver samples in tray 1 and 2, however we have 32 samples in total in both plates. Are they duplicated samples?

Also we are not sure about the DNA samples in plate 7? Could you please clarify?

Thank you

[REDACTED]

**From:** Kaukinen, Karia [mailto:Karia.Kaukinen@dfo-mpo.gc.ca]  
**Sent:** Thursday, May 16, 2019 3:27 PM  
**To:** [REDACTED] Schulze, Angela <Angela.Schulze@dfo-mpo.gc.ca>; Li, Shaorong <Shaorong.Li@dfo-mpo.gc.ca>  
**Cc:** DiCicco, Emiliano <Emiliano.DiCicco@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Ming, Tobi <Tobi.Ming@dfo-mpo.gc.ca>; [REDACTED]  
**Subject:** RE: Samples for BC CAHS Bench Validation

Hi,  
Since you did not include a time, I cannot confirm the exact person. However, between the hours of 5am and usually 6pm, there are people within our group in the lab. I am assuming that [REDACTED] will leave Campbell River in the morning arriving here by approximately 10am? I will notify the commissionaire that we are expecting him and to call up to one or more of us to ensure [REDACTED] turn around time is quick.  
Have a great long weekend,  
Karia

s.19(1)

---

**From:** [REDACTED]  
**Sent:** 16 May 2019 16:34  
**To:** Kaukinen, Karia; Schulze, Angela; Li, Shaorong

**Cc:** DiCicco, Emiliano; Miller-Saunders, Kristi; Ming, Tobi; [REDACTED]  
**Subject:** RE: Samples for BC CAHS Bench Validation

Hello Karia,

Thank you for the quick reply. [REDACTED] will drive to Nanaimo on Tuesday (May 21<sup>st</sup>) to pick up the samples. He will have the cooler and dry ice for immediate return to CAHS.

Please confirm that someone will be available to meet [REDACTED] on Tuesday.

Regards,

**From:** Kaukinen, Karia <Karia.Kaukinen@dfo-mpo.gc.ca>

**Sent:** May 16, 2019 12:17 PM

**To:** [REDACTED]; Schulze, Angela <Angela.Schulze@dfo-mpo.gc.ca>; Li, Shaorong <Shaorong.Li@dfo-mpo.gc.ca>

**Cc:** DiCicco, Emiliano <Emiliano.DiCicco@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Ming, Tobi <Tobi.Ming@dfo-mpo.gc.ca>

**Subject:** RE: Samples for BC CAHS Bench Validation

Hi,

The sample trays are all in a clearly labelled box ready to go. If you are going to have someone pick them up, they will need dry ice and cooler. Cooler large enough to accommodate the box measuring 8.5" wide, 6" tall and 5" deep and approximately 10lbs of dry ice (if returning immediately to CAHS), otherwise 25 lbs of dry ice for less direct transport.  
Karia

**From:** [REDACTED]

**Sent:** May-16-19 11:26 AM

**To:** Kaukinen, Karia <Karia.Kaukinen@dfo-mpo.gc.ca>; Schulze, Angela <Angela.Schulze@dfo-mpo.gc.ca>; Li, Shaorong <Shaorong.Li@dfo-mpo.gc.ca>

**Subject:** Samples for BC CAHS Bench Validation

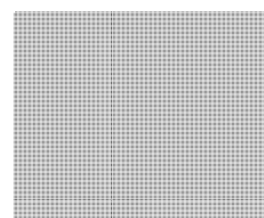
Hello,

My name is [REDACTED] here at BC CAHS in Campbell River. I understand we are to coordinate the shipping of 20 samples to Campbell River for RT-qPCR screening for PRV.

Please let me know when the samples are ready for shipment or pickup, whichever is easier and more convenient for you.

Kind regards,

s.19(1)



BC Centre for Aquatic Health Sciences

Street Address: 871A Island Hwy, Campbell River, BC  
Mailing Address: PO Box 25070 Tyee, Campbell River, BC, Canada V9W 0B7  
ph: 250 286-6102 f: 250 286-6103  
email: [REDACTED]  
web: [www.cahs-bc.ca](http://www.cahs-bc.ca)

s.19(1)

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<https://twitter.com/BCCAHS>

<https://www.linkedin.com/company/bc-centre-for-aquatic-health-sciences-society>

***BC CAHS Celebrating 14 Years 2005 - 2019***

No further information has been removed or severed from this page



**Miller-Saunders, Kristi**

---

**From:** Higgins, Mark  
**Sent:** May-23-19 12:10 PM  
**To:** MacDougall, Lesley; Miller-Saunders, Kristi  
**Subject:** RE: [REDACTED]  
**Attachments:** [REDACTED]

My comments and changes included. Mark.

---

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** May-23-19 9:01 AM  
**To:** Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Subject:** FW: [REDACTED]

Hi both – all the court response stuff is flying around furiously now (the bad news), but there has been agreement to hold off on work on the practitioner's guide until we get some of the other components nailed down a bit more (so a bit of good news).

Unfortunately, that means we're looking for your thoughts on this one pager TODAY, if you can do so.

Thanks in advance;  
Lesley

---

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** May-23-19 8:15 AM  
**To:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Subject:** Fw: [REDACTED]

s.21(1)(a)  
s.21(1)(b)  
s.23

Sent from my BlackBerry 10 smartphone on the Bell network.

---

**From:** Webb, Allison <Allison.Webb@dfo-mpo.gc.ca>  
**Sent:** Wednesday, May 22, 2019 21:30  
**To:** Dostal, Alexandra; MacDougall, Lesley; Lowe, Carmel; Sharzer, Stephen (DOJ); House, Matthew (DOJ); Reid, Rebecca; Campbell, John P.; McPherson, Arran; Haesevoets, Roderick; Parsons, Jay; Moore, Wayne  
**Subject:** [REDACTED]

Please find attached [REDACTED] There are a few areas that require additional work. For consideration and comments.

Regards,  
Allison

Allison Webb, Director / Directrice  
Aquaculture Management / Gestion de l'aquaculture

Fisheries Management Branch / Direction de la gestion des pêches

Fisheries and Oceans Canada / Pêches et Océans Canada

200 - 401 Burrard St / Rue Burrard, Vancouver BC / C.B. V6C 3S4 Canada

604-666-7009

[Allison.webb@dfo-mpo.gc.ca](mailto:Allison.webb@dfo-mpo.gc.ca)

---

**From:** Dostal, Alexandra <[Alexandra.Dostal@dfo-mpo.gc.ca](mailto:Alexandra.Dostal@dfo-mpo.gc.ca)>

**Sent:** Wednesday, May 22, 2019 12:37 PM

**To:** Webb, Allison <[Allison.Webb@dfo-mpo.gc.ca](mailto:Allison.Webb@dfo-mpo.gc.ca)>; MacDougall, Lesley <[Lesley.MacDougall@dfo-mpo.gc.ca](mailto:Lesley.MacDougall@dfo-mpo.gc.ca)>; Lowe, Carmel <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>; Sharzer, Stephen (DOJ) <[stephen.sharzer@justice.gc.ca](mailto:stephen.sharzer@justice.gc.ca)>; House, Matthew (DOJ) <[Matthew.House@justice.gc.ca](mailto:Matthew.House@justice.gc.ca)>; Reid, Rebecca <[Rebecca.Reid@dfo-mpo.gc.ca](mailto:Rebecca.Reid@dfo-mpo.gc.ca)>; Campbell, John P. <[John.Campbell@dfo-mpo.gc.ca](mailto:John.Campbell@dfo-mpo.gc.ca)>; McPherson, Arran <[Arran.McPherson@dfo-mpo.gc.ca](mailto:Arran.McPherson@dfo-mpo.gc.ca)>; Haesevoets, Roderick <[Roderick.Haesevoets@dfo-mpo.gc.ca](mailto:Roderick.Haesevoets@dfo-mpo.gc.ca)>; Parsons, Jay <[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca)>; Hubley, Marian <[Marian.Hubley@dfo-mpo.gc.ca](mailto:Marian.Hubley@dfo-mpo.gc.ca)>

**Subject:** Follow up to call

Hello colleagues,

I wanted to follow up on our conversation today after the DM meeting to make sure we all know who is responsible for what. We will meet tomorrow morning if you are available – I have sent out an invite for 8am Pacific time.

1. Guidance – Science to update with intro part and go through and review and change as per call today (AMD to support)
2. Decision note – draft circulated tomorrow am (AMD)
3. 1 pager on research (Science)
4. 1 pager on testing (Pacific)
5. Process note on next steps with 'Namgis, advisory committee and a potential "SRKW type" table. (AMD and Pacific)
6. Comms package (Comms)

Let me know what I am missing!

Cheers,

Alix

**Alix Dostal**

Director General, Aquaculture Management Directorate | Directrice générale, Direction de la gestion de l'aquaculture

Aquaculture Management Directorate | Direction de la gestion de l'aquaculture

Telephone | Téléphone: 613-993-1884

[Alexandra.Dostal@dfo-mpo.gc.ca](mailto:Alexandra.Dostal@dfo-mpo.gc.ca)

Government of Canada | Gouvernement du Canada

**Pages 720 to / à 724  
are withheld pursuant to sections  
sont retenues en vertu des articles**

**21(1)(b), 23, 21(1)(a)**

**of the Access to Information Act  
de la Loi sur l'accès à l'information**

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** May-23-19 12:23 PM  
**To:** MacDougall, Lesley; Higgins, Mark  
**Subject:** RE: [REDACTED]  
**Attachments:** [REDACTED]

Here are my comments  
Kristi

---

**From:** MacDougall, Lesley  
**Sent:** May 23, 2019 9:00 AM  
**To:** Higgins, Mark; Miller-Saunders, Kristi  
**Subject:** FW: [REDACTED]

Hi both – all the court response stuff is flying around furiously now (the bad news), but there has been agreement to hold off on work on the practitioner's guide until we get some of the other components nailed down a bit more (so a bit of good news).

Unfortunately, that means we're looking for your thoughts on this one pager TODAY, if you can do so.

Thanks in advance;  
Lesley

---

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** May-23-19 8:15 AM  
**To:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Subject:** Fw: [REDACTED]

s.21(1)(a)  
s.21(1)(b)  
s.23

Sent from my BlackBerry 10 smartphone on the Bell network.

---

**From:** Webb, Allison <Allison.Webb@dfo-mpo.gc.ca>  
**Sent:** Wednesday, May 22, 2019 21:30  
**To:** Dostal, Alexandra; MacDougall, Lesley; Lowe, Carmel; Sharzer, Stephen (DOJ); House, Matthew (DOJ); Reid, Rebecca; Campbell, John P.; McPherson, Arran; Haesevoets, Roderick; Parsons, Jay; Moore, Wayne  
**Subject:** [REDACTED]

Please find attached a draft paper on testing. There are a few areas that require additional work. For consideration and comments.

Regards,  
Allison

Allison Webb, Director / Directrice  
Aquaculture Management / Gestion de l'aquaculture

Fisheries Management Branch / Direction de la gestion des pêches

Fisheries and Oceans Canada / Pêches et Océans Canada

200 - 401 Burrard St / Rue Burrard, Vancouver BC / C.B. V6C 3S4 Canada

604-666-7009

[Allison.webb@dfo-mpo.gc.ca](mailto:Allison.webb@dfo-mpo.gc.ca)

---

**From:** Dostal, Alexandra <[Alexandra.Dostal@dfo-mpo.gc.ca](mailto:Alexandra.Dostal@dfo-mpo.gc.ca)>

**Sent:** Wednesday, May 22, 2019 12:37 PM

**To:** Webb, Allison <[Allison.Webb@dfo-mpo.gc.ca](mailto:Allison.Webb@dfo-mpo.gc.ca)>; MacDougall, Lesley <[Lesley.MacDougall@dfo-mpo.gc.ca](mailto:Lesley.MacDougall@dfo-mpo.gc.ca)>; Lowe, Carmel <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>; Sharzer, Stephen (DOJ) <[stephen.sharzer@justice.gc.ca](mailto:stephen.sharzer@justice.gc.ca)>; House, Matthew (DOJ) <[Matthew.House@justice.gc.ca](mailto:Matthew.House@justice.gc.ca)>; Reid, Rebecca <[Rebecca.Reid@dfo-mpo.gc.ca](mailto:Rebecca.Reid@dfo-mpo.gc.ca)>; Campbell, John P. <[John.Campbell@dfo-mpo.gc.ca](mailto:John.Campbell@dfo-mpo.gc.ca)>; McPherson, Arran <[Arran.McPherson@dfo-mpo.gc.ca](mailto:Arran.McPherson@dfo-mpo.gc.ca)>; Haesevoets, Roderick <[Roderick.Haesevoets@dfo-mpo.gc.ca](mailto:Roderick.Haesevoets@dfo-mpo.gc.ca)>; Parsons, Jay <[Jay.Parsons@dfo-mpo.gc.ca](mailto:Jay.Parsons@dfo-mpo.gc.ca)>; Hubley, Marian <[Marian.Hubley@dfo-mpo.gc.ca](mailto:Marian.Hubley@dfo-mpo.gc.ca)>

**Subject:** Follow up to call

Hello colleagues,

I wanted to follow up on our conversation today after the DM meeting to make sure we all know who is responsible for what. We will meet tomorrow morning if you are available – I have sent out an invite for 8am Pacific time.

1. Guidance – Science to update with intro part and go through and review and change as per call today (AMD to support)
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4. 1 pager on testing (Pacific)
5. Process note on next steps with 'Namgis, advisory committee and a potential "SRKW type" table. (AMD and Pacific)
6. Comms package (Comms)

Let me know what I am missing!

Cheers,

Alix

**Alix Dostal**

Director General, Aquaculture Management Directorate | Directrice générale, Direction de la gestion de l'aquaculture  
Aquaculture Management Directorate | Direction de la gestion de l'aquaculture

Telephone | Téléphone: 613-993-1884

[Alexandra.Dostal@dfo-mpo.gc.ca](mailto:Alexandra.Dostal@dfo-mpo.gc.ca)

Government of Canada | Gouvernement du Canada

**Pages 727 to / à 730  
are withheld pursuant to sections  
sont retenues en vertu des articles**

**21(1)(b), 23, 21(1)(a)**

**of the Access to Information Act  
de la Loi sur l'accès à l'information**

**From:** [REDACTED]  
**Sent:** May-24-19 11:34 AM  
**To:** Miller-Saunders, Kristi; [REDACTED]  
**Subject:** Fwd: PRV Science Advisory Report now published

Begin forwarded message:

**s.19(1)**

"tony.farrell@ubc.ca<mailto:tony.farrell@ubc.ca>" <tony.farrell@ubc.ca<mailto:tony.farrell@ubc.ca>>,  
 [REDACTED]  
 "Gary.Marty@gov.bc.ca<mailto:Gary.Marty@gov.bc.ca>" <Gary.Marty@gov.bc.ca<mailto:Gary.Marty@gov.bc.ca>>,

"Boily, France"

<France.Boily@dfo-mpo.gc.ca<mailto:France.Boily@dfo-mpo.gc.ca>>

Subject: PRV Science Advisory Report now published

Hello CSAS PRV Participants,

I would like, once again, to thank all of you for your participation in the assessment of risk to Fraser River Sockeye Salmon due to piscine orthoreovirus transfer from Atlantic Salmon farms in the Discover Islands area, British Columbia and your input on the final version of the CSAS Science Advisory Report (SAR).

The PRV SAR from the CSAS meeting is now available on the DFO website and the two expected research documents will be following in the upcoming weeks.

Link to publication:

[http://www.dfo-mpo.gc.ca/csas-sccs/Publications/SAR-AS/2019/2019\\_022-eng.html](http://www.dfo-mpo.gc.ca/csas-sccs/Publications/SAR-AS/2019/2019_022-eng.html)

Thank you again for your time throughout this process.

Jay

Jay Parsons, PhD

Director

Aquaculture, Biotechnology and Aquatic Animal Health Sciences Branch Fisheries and Oceans Canada / Government of Canada

200 Kent Street, Stn 12E239 Ottawa, ON Canada K1A 0E6 Jay.Parsons@dfo-mpo.gc.ca/<mailto:Jay.Parsons@dfo-mpo.gc.ca/> Tel. 613-990-0278

Directeur

Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques Pêches et Océans Canada / Gouvernement du Canada

200 Kent Street, Stn 12E239 Ottawa, ON Canada K1A 0E6 Jay.Parsons@dfo-mpo.gc.ca<mailto:Jay.Parsons@dfo-mpo.gc.ca> / Tél. 613-990-0278

<image001.png>

s.19(1)



## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** May-24-19 1:18 PM  
**To:** Miller-Saunders, Kristi; [REDACTED]  
**Subject:** Re: PRV and the CSAS review process and public messaging by the DFO

Thanks Kristi ... to which the response should be ... for the working papers they (Garver and Polinski and the rest of the people involved in putting together the risk assessment) relied on incomplete, unpublished studies and incomplete data for that assessment.

If the uncertainties and data gaps have been since closed and the working papers are still in draft form ... IMO, the PRV assessment panel should be re-convened to address all this new information ... especially given the high profile this pathogen presents .. and make sure the final papers and conclusions are based on up-to-date information.

---

**From:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Sent:** Friday, May 24, 2019 12:51 PM  
**To:** [REDACTED]  
**Subject:** RE: PRV and the CSAS review process and public messaging by the DFO

While I was not at the meeting in question, I did ascertain from others that were whether these statements were indeed made, and they concurred. Hence I believe that is reasonable to ask these questions of those involved in the production of the reports for the CSAS. I can guess that they will say that the data were not available at the time the reports were generated (which would have been before January), but I guess we will see.

Kristi

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**From:** [REDACTED]  
**Sent:** May 24, 2019 12:42 PM  
**To:** [REDACTED] Miller-Saunders, Kristi; [REDACTED]  
**Subject:** Fw: PRV and the CSAS review process and public messaging by the DFO

For your information.

Please see the email below sent to senior bureaucrats at the CSAS and the Minister of Fisheries and Oceans Canada.

---

**From:** [REDACTED]  
**Sent:** Friday, May 24, 2019 12:27 PM  
**To:** Parsons, Jay; Olivier, Gilles; Craig Stephen  
**Cc:** Jonathan.Wilkinson@parl.gc.ca  
**Subject:** PRV and the CSAS review process and public messaging by the DFO

Hi Jay/Gilles/Craig.

CC: The Honourable Jonathan Wilkinson, Minister, Fisheries and Oceans Canada

[REDACTED]

I have some concerns I would like to share concerning information being circulated publicly by DFO scientists about the presence/prevalence of PRV in Pacific waters and how this virus may be spread. This information was revealed at the recent Aquaculture Association of Canada's conference held in Victoria May 5-8, 2019.

To the best of my knowledge, none of the information being shared publicly (by DFO scientists and/or scientists working with the DFO who presented at this conference) — and mentioned below — was made available to the experts who participated in the recent (January 29-31, 2019) CSAS PRV risk assessment. That gives me cause for concern.

At this conference it was asserted that (individual points follow below in bullet form):

- Atlantic salmon hatcheries in B.C. are now free of PRV

I find this difficult to believe given that, during the PRV risk assessment held only a few months ago, we were presented with data that showed:

***At line 270 in the risk assessment:*** "Between 2013 and 2018, PRV has been detected in hatcheries in all years..." Granted, the data showed a reduction in the percentage of positive fish over time, but it still showed that in every case, PRV was detected.

It was also noted that these were data made available to DFO from industry in January 2019.

There were no DFO test data provided/presented to support or challenge these findings. Nor were we advised that DFO had an active surveillance program in place to look for PRV in hatcheries. If anything, we were told there were no requirements in place for industry (or DFO) to test hatchery fish for PRV prior to transfer of fish to marine sites. Now, only three short months later, we have DFO scientists standing in front of an international audience telling them that all Atlantic salmon hatcheries in B.C. are PRV-free. How can that be?

It is my current understanding that DFO has not been testing hatcheries for this pathogen, so it would be helpful to know where are these new data come from. It is challenging to accept that the world of hatchery biosecurity can change so rapidly in just three months (since the close of the CSAS) when this pathogen (PRV) was prevalent in all Atlantic salmon hatcheries tested for the past five years.

- PRV (B.C.) came first to New Brunswick and from there to B.C. in about 1950-1960. It was suggested that attempts to plant Atlantics on the West Coast were the vector for transmission of the virus to B.C. (1905-1935).

It would seem this information has only come to light in the past three months. Not once was it mentioned in any of the working papers that were up for peer review during the CSAS process that this virus came to this coast from the East Coast of Canada (let alone that it came from New Brunswick) following attempts to establish Atlantic salmon here. This conclusion actually diverges from the published literature. On what credible evidence that was not available to the CSAS are DFO scientists now convinced this is the case?

- PRV levels are easily measured in the water. Samples have been taken. **All near-farm samples tested positive for PRV @ at least 10,000 copies per litre.**

As I recall, none of this information or data was included in any of the working papers presented to the CSAS peer-review panel. I find it hard to believe that DFO has since mobilized crews to go to every salmon farm in B.C.; plan and conduct statistically significant sampling in the waters near those farms; and, have those samples gathered, processed and the data analyzed in just three months. Is that the case or is DFO somehow relying on data that should have been available to the CSAS?

Were any of these data in hand with the DFO prior to January 2019? If so, why were they excluded from any of the papers, commentary or analysis during the PRV risk assessment? As I recall, we were told that there were no data concerning concentrations of PRV particles in waters in or around farms — NONE. Therefore, assumptions, calculations and modelling similar to what was carried out for other pathogens in other risk-assessment scenarios could not be done for this pathogen (unlike during prior risk assessments for other pathogens — like *Aeromonas salmonicida* — wherein concentrations of viral particles outside of, and distant to, farms could be estimated and modelled using Mike Forman's particle dispersion models).

- 350 species have been checked to see if they might be a “reservoir species” for PRV, and they were all found to be clean. (Note: no species list was provided to back up this assertion.)

This is highly improbable. A recent CSAS assessment was provided a list of ecological conservation priority species for MPA network design. It was included as an appendix in a CSAS report from June 2017 and includes fish, marine mammals, invertebrates, plants and algae. That list only mentions 142 species. It includes salmonids, flat fishes, forage fishes, rockfishes, ground fishes, sharks, pinnipeds and whales, among others.

I think it would be impossible to find, identify and sample 350 separate species and have them tested for PRV in just three months. There was no mention at any time during the CSAS review of any DFO surveillance or testing program where attempts were being made to find a “reservoir” species for this virus. I think it is reasonable to ask where this information came from. How long has the DFO had this information in hand? If they had any of this information prior to January 2019 (a logical conclusion would be that they did, at least in part), why was it not shared during the CSAS review?

It may be that what was transmitted to the public at this conference was wrongly interpreted. It may be that the actual assertion is that 350 “SAMPLES” have been taken in an attempt to identify a “reservoir species” and all of them were clean. However, even that would be a stretch. During the CSAS PRV review, we were presented with data showing testing in 10 Pacific fish species (salmon, trout and eulachon). **All of them tested**

**positive for PRV.** However, none were alleged to be a “reservoir species.” They were all identified as “fish species in which PRV genetic material was detected.”

I question how any scientist can say then that all species (samples?) tested for PRV were “clean.” Yet this is the message your scientists are sending to the world.

- They have benthic samples but haven’t yet checked them out as a potential reservoir.

Again, there was no mention at any time during the CSAS review of any DFO surveillance or testing program where attempts were being made to find a “reservoir” for this virus. Surely this information, or at least the possibility that it was being gathered, would/should have been “known” prior to January 2019. But it was not even mentioned.

- The logical conclusion is that the wild fish have PRV and they’re infecting the farmed salmon.

Apparently, this was an assertion made publicly at this conference by one of our fellow CSAS experts during one of the post-presentation question/discussion sessions. I don’t recall that we ever reached that kind of conclusion during the CSAS review — not even close. Yet this is what is being reported to me as having been said by DFO in public forums.

- Tank and ocean pen experiments were conducted with Atlantic salmon at DFO’s lab at Departure Bay (in West Vancouver) to see if the fish would contract the virus. Apparently, none of the fish became infected.

This seems entirely inconsistent with the assertion made above: that wild salmon are the main vector for transmission of the virus. The waters in and around Departure Bay are used by both juvenile and wild adult Pacific salmon almost year-round. If it were true that wild salmon are the key vector for transmission of the virus, the experimental fish would have become infected just as easily as the fish confined to fish farms. Again, none of these study results (or even the mention of such exposure trials) were mentioned during the CSAS review. Why is that?

I am sharing this with you and the minister because it troubles me, as a scientist who participated in the CSAS peer-review on the assessment of risk of exposure to wild Fraser River sockeye salmon from PRV on fish farms, that many of the unknowns and uncertainties that were identified in that review are now being portrayed publicly by DFO scientists as “known information.” And I find it impossible to believe these large information gaps could have been filled in such a short time.

We are concerned as well that the minister may be getting incorrect, or questionable, information that he may use to guide DFO’s evolving PRV policy, which is to be publicly announced in the near future. For that reason I have CC’d him on this email.

I would appreciate a reply from the CSAS to these concerns as they are, in our opinion, valid and need to be addressed.

I would also ask that you share this submission with the entire PRV review panel (Steering Committee and participants) so that they can share their opinions on these matters.

Regards

s.19(1)



David Suzuki Foundation

No further information has been removed or severed from this page

## Miller-Saunders, Kristi

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**From:** Higgins, Mark  
**Sent:** June-03-19 8:57 AM  
**To:** MacDougall, Lesley; Miller-Saunders, Kristi  
**Subject:** RE: your early thoughts - proposed research questions

Lesley, Work is already being done as outlined by Kyle on mapping out strains of the PRV on the coast and elsewhere under Stewarts work. I would suggest that it is much more effective to adapt management strategies to minimize PRV infection in out going smolts like Kristi suggests, through double or triple washing of eggs (which I think SEP has now adopted, and the industry has been doing for some time). These actions along with current management strategies like the audit program, mandatory reporting under conditions of licence on the changes in health at the farm level, and veterinary oversight will provide DFO with information if there is a change in the mortality or morbidity on farms that could be predictive of a change disease status due to changing environmental conditions.

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** May-31-19 8:45 AM  
**To:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>  
**Subject:** your early thoughts - proposed research questions

Hi Kristi and Mark – as discussed briefly earlier, NHQ is interested in our thoughts on the attached document, outlining a proposed research project/direction. The intent is for this project to be departmental research *in addition to* the collaborative research being contemplated with the Broughton group, not replacing it.

Still early days, but I would like your take on what might be feasible, how long a project would we be looking at, what kinds of studies would be needed, does this dovetail into work we're already doing, if you have ballpark costs – financial and human resources, etc., would this create pressures elsewhere in your sections? Since we're still at 'kicking tires' phase a super detailed accounting isn't needed, but any general impressions, obvious costs, areas of concern, areas of overlap/benefit that you can identify would be great. If possible, by today or Monday...

Thanks  
Lesley

Lesley MacDougall  
A/Division Manager, Aquatic Diagnostics, Genomics & Technology / Division des diagnostics, la génomique, de la technologie aquatique  
Fisheries and Oceans Canada / Pêches et Océans Canada  
Pacific Biological Station / Station Biologique du Pacifique  
Nanaimo, B.C. V9T 6N7  
250-756-7395  
[Lesley.MacDougall@dfo-mpo.gc.ca](mailto:Lesley.MacDougall@dfo-mpo.gc.ca)

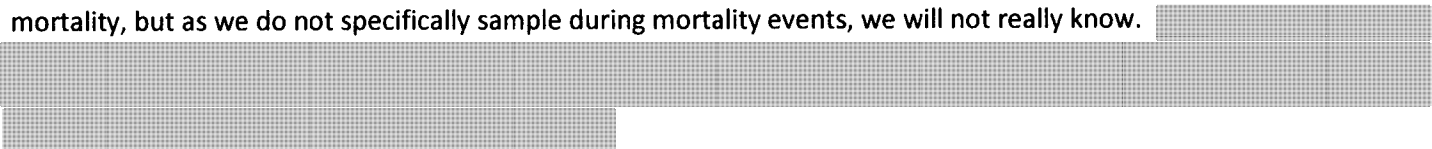
## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** June-03-19 3:23 PM  
**To:** MacDougall, Lesley; Higgins, Mark  
**Subject:** RE: Proposed PRV research study

I just read Mark's response and I thought I would add a bit more on this.

First, I disagree that the audit program, in its present form, will really add a lot of insight into whether PRV/HSMI or PRV/Jaundice becomes more of an issue in future. In my view, if this program continues to "not recognize" the pathological lesions that are diagnostic of HSMI, or the lesion patterns that have defined PRV-related diseases in Pacific salmon around the world, including farmed Chinook salmon (which additionally shows clinical signs of jaundice at a final stage of the disease), then they will likely continue to not recognize these diseases. Moreover, if they concentrate their efforts towards recognizing diseases only on farms that meet the trigger threshold of mortality (something like 2% of fish in 24 hours or 4-5% over 5 days (?), diseases like both of these, which are chronic in nature and may induce mortality at a low level over a month or longer (3 months for Jaundice), they are unlikely to recognize if there is substantive change in disease "impact". Moreover, it is highly probable that these diseases may be missed as they occur as co-infections when other diseases, like BKD, are manifesting; the occurrence of PRV-related lesions is likely masked by these co-infections, as typically vets recognize the disease that they are most familiar with. We certainly see this issue in the audit data. In this case, as co-infections, these diseases may very well manifest during elevated mortality, but as we do not specifically sample during mortality events, we will not really know.



As for budgeting on getting a better handle on the fallowing issue and the farm-farm connectivity, as we have discussed, I believe that work should be conducted using the multi-agent high throughput monitoring approach rather than simply a focus on PRV, and would take 2-3 years of concerted effort in collaboration with hydrographic modeling. I would be happy to prepare a proposal on this in future if there is interest. The AMD has had interest, but Ottawa has not put this information high on the priority list. I can think of little else that would be a higher priority, except perhaps sea lice resistance to SLICE, implementation of semi-closed containment farms in high risk areas, and identification of critical ocean habitats for early marine rearing of stocks of conservation concern where farms are currently operational or are looking to move. The Genome Canada project, if funded, will delve into the last on this list.

Kristi

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

s.21(1)(a)

s.21(1)(b)

**From:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Sent:** May-31-19 9:41 AM  
**To:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>; Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>  
**Subject:** RE: Proposed PRV research study

Leslie,

While I am all for continued monitoring using high throughput technologies as well as traditional approaches to disease assessments are needed to ensure that we do not miss changes in trends or disease occurrence, I think there are relatively easy measure to put into place NOW that would further minimize these risks.

First, as it appears that double or triple disinfection of the eggs has worked well in significantly decreasing the risk of transmission of PRV and possibly other viruses in freshwater hatcheries (e.g. very low level of coronavirus in Quinsam Chinook this year). This practice should become a requirement for aquaculture and SEP hatcheries, and we should track how well the procedure minimizes levels of viral infection.

Second, it is clear to me that fallowing is likely a main issue in the rapid reinfection of naïve fish moved to salmon net pens, given that at least for PRV, we do not see the same pattern of high infection rates in the Strait of Georgia. Conducting research that would provide empirical evidence to optimise fallowing times is a start,

and a better understanding of connectivity between farms is paramount to establish boundaries of areas. I know that some very useful work has been done with hydrodynamic models in the Discovery Islands and this should be expanded to other areas, but I believe needs to be additionally populated with eDNA data on Atlantic salmon and infectious agents in these areas, as we discussed in our meeting with the Namgis. This could provide a key solution to reducing infections of a large range of agents that accumulate around high density farming areas.

I think being able to announce new policies on the treatment of eggs as a proactive measure to reduce the continuous infection cycles on farms and burdens of infective agents that could be transmissible to wild fish would be seen positively by the public. It gets to the issues more broadly and gets the department away from always being on the defensive on why they are not taking action on virus x (in this case PRV, but in future it could be something else). This would be a truly precautionary approach in my view, and a good start towards appeasing the concerned public.

Kristi

---

**From:** MacDougall, Lesley  
**Sent:** May 31, 2019 8:01 AM  
**To:** Higgins, Mark; Miller-Saunders, Kristi  
**Subject:** FW: Proposed PRV research study

Hi Mark and Kristi

Kristi – this is the proposed other work that is being kicked around by the ADM, I mentioned it yesterday in our meeting. My hope was that the collaborative work that is being sketched out right now with the Broughton FN would be our priority, but this suggested work is something that the ADM potentially sees taking place in parallel apparently.

When I first read the description of the work my comment to Carmel was I think it would be very difficult to provide advice on potential changes to virulence with potential links to environmental conditions. I also noted that with my limited understanding of several projects (Kyle's work as well as Kristi's) I thought we were already trying to get at some of these questions but scoped in a more manageable way (e.g. PRV transmission, potential study of pristine vs. industrialized sites and differences in stress responses in each etc).

However, this proposed research isn't going away. I don't have any specific request for your response – YET – but please review the attached document...and expect that there will be some 'ask' coming in the near future....

Thanks  
Lesley

**From:** Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>  
**Sent:** May-29-19 8:00 AM

s.21(1)(a)  
s.21(1)(b)



**To:** Lowe, Carmel <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>; MacDougall, Lesley <[Lesley.MacDougall@dfo-mpo.gc.ca](mailto:Lesley.MacDougall@dfo-mpo.gc.ca)>  
**Cc:** McPherson, Arran <[Arran.McPherson@dfo-mpo.gc.ca](mailto:Arran.McPherson@dfo-mpo.gc.ca)>; Moore, Wayne <[Wayne.Moore@dfo-mpo.gc.ca](mailto:Wayne.Moore@dfo-mpo.gc.ca)>; McGill, Stephanie <[Stephanie.McGill@dfo-mpo.gc.ca](mailto:Stephanie.McGill@dfo-mpo.gc.ca)>  
**Subject:** Proposed PRV research study  
**Importance:** High

Carmel, Lesley,

As part of the approach to moving forward with the response to the decision around future movements of fish and the re-consideration of the "PRV policy", we are proposing that some additional research be undertaken. I am attaching a short, high level perspective on some possible future research and we would like to discuss this with you further and in particular seek your perspective on what a more detailed approach might look like.

We will set up a call to discuss further.

Thank you, Jay

No information has been removed or severed from this page

## Miller-Saunders, Kristi

---

**From:** Schulze, Angela  
**Sent:** June-04-19 2:27 PM  
**To:** Gideon Mordecai (gmordecai@eoas.ubc.ca)  
**Cc:** [REDACTED] Miller-Saunders, Kristi; DiCicco, Emiliano  
**Subject:** PRV new paper  
**Attachments:** Dhamotharan\_et\_al\_2019\_Evolution of PRV Genome linked to emergence of HSMI in farmed salmon.pdf

Hey I found a new paper looking a sequence variation in PRV, just in case you guys have not seen it yet. They suggest we need more sequences from wild Atlantic salmon at the end Amy!





Angela

*Angela Schulze  
Molecular Genetics Laboratory  
Pacific Biological Station  
Nanaimo, B.C.  
V9K 6N7  
250-756-3357  
[Angela.Schulze@dfo-mpo.gc.ca](mailto:Angela.Schulze@dfo-mpo.gc.ca)*

s.19(1)

## Article

# Evolution of the *Piscine orthoreovirus* Genome Linked to Emergence of Heart and Skeletal Muscle Inflammation in Farmed Atlantic Salmon (*Salmo salar*)

Kannimuthu Dhamotharan <sup>1</sup>, Torstein Tengs <sup>2</sup>, Øystein Wessel <sup>1</sup>, Stine Braaen <sup>1</sup>, Ingvild B. Nyman <sup>1</sup>, Elisabeth F. Hansen <sup>1</sup>, Debes H. Christiansen <sup>3</sup>, Maria K. Dahle <sup>4</sup>, Espen Rimstad <sup>1,\*</sup> and Turhan Markussen <sup>1</sup>

<sup>1</sup> Faculty of Veterinary Medicine, Norwegian University of Life Sciences, 0454 Oslo, Norway; dhamokan@nmbu.no (K.D.); oystein.wessel@nmbu.no (Ø.W.); stine.braaen@nmbu.no (S.B.); ingvild.nyman@nmbu.no (I.B.N.); elisabeth.hansen@nmbu.no (E.F.H.); turhan.markussen@nmbu.no (T.M.)

<sup>2</sup> Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, 1433 Ås, Norway; torstein.tengs@nmbu.no

<sup>3</sup> Faroese Food and Veterinary Authority, National Reference Laboratory for Fish Diseases, FO-110 Tórshavn, Faroe Islands; debesc@hfs.fo

<sup>4</sup> Department of Immunology, Norwegian Veterinary Institute, 0454 Oslo, Norway; maria.dahle@vetinst.no

\* Correspondence: espen.rimstad@nmbu.no; Tel.: +47-672-32-227

Received: 27 March 2019; Accepted: 20 May 2019; Published: 22 May 2019



**Abstract:** Heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon (*Salmo salar*) was first diagnosed in Norway in 1999. The disease is caused by *Piscine orthoreovirus*-1 (PRV-1). The virus is prevalent in farmed Atlantic salmon, but not always associated with disease. Phylogeny and sequence analyses of 31 PRV-1 genomes collected over a 30-year period from fish with or without HSMI, grouped the viral sequences into two main monophylogenetic clusters, one associated with HSMI and the other with low virulent PRV-1 isolates. A PRV-1 strain from Norway sampled in 1988, a decade before the emergence of HSMI, grouped with the low virulent HSMI cluster. The two distinct monophylogenetic clusters were particularly evident for segments S1 and M2. Only a limited number of amino acids were unique to the association with HSMI, and they all located to S1 and M2 encoded proteins. The observed co-evolution of the S1-M2 pair coincided in time with the emergence of HSMI in Norway, and may have evolved through accumulation of mutations and/or segment reassortment. Sequences of S1-M2 suggest selection of the HSMI associated pair, and that this segment pair has remained almost unchanged in Norwegian salmon aquaculture since 1997. PRV-1 strains from the North American Pacific Coast and Faroe Islands have not undergone this evolution, and are more closely related to the PRV-1 precursor strains not associated with clinical HSMI.

**Keywords:** PRV-1; *Piscine orthoreovirus*; HSMI; virulence; reassortment; viral evolution

## 1. Introduction

Atlantic salmon (*Salmo salar*) aquaculture is a significant food production industry. Farmed fish are kept at high rearing densities, and outbreaks of infectious diseases strongly impact productivity and economic output [1]. Heart and skeletal muscle inflammation (HSMI) was reported for the first time in 1999, occurring during the seawater production phase in farmed Atlantic salmon in Mid-Norway [2], and rapidly emerged as an important disease. A few years later, disease outbreaks were reported from farms all along the Norwegian coast, and a maximum number of 181 outbreaks were registered in 2014,

**Pages 744 to / à 758**  
**are withheld pursuant to section**  
**sont retenues en vertu de l'article**

**68(a)**

**of the Access to Information Act**  
**de la Loi sur l'accès à l'information**

## Miller-Saunders, Kristi

---

**From:** Pacific Salmon Foundation [REDACTED]  
**Sent:** June-06-19 2:20 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** PSF Statement: Regarding DFO decision for aquaculture salmon testing

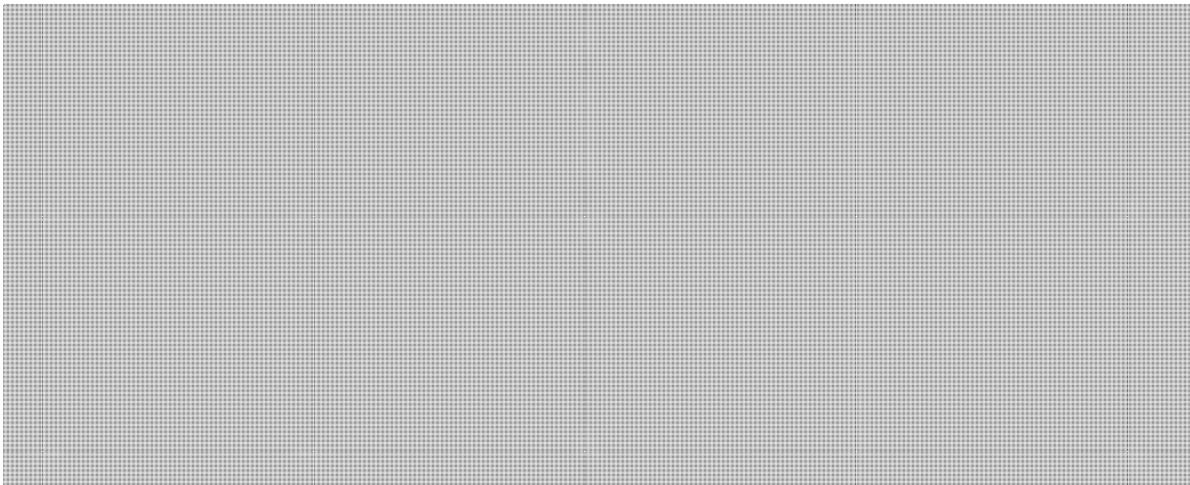
Available at: <https://www.psf.ca/news-media/statement-psf-president-and-ceo-michael-meneer>



As a friend of the Pacific Salmon Foundation who cares for wild salmon, you may have read the headlines yesterday announcing DFO's decision to strengthen certain testing and reporting measures on salmon farms. Please read our statement below:

### **Statement of PSF President and CEO Michael Meneer**

*Regarding decision to test for Piscine Orthoreovirus (PRV) in  
aquaculture salmon*



s.19(1)

s.68(a)

s.68(a)

Pacific Salmon Foundation  
300 - 1682 West 7th Avenue British Columbia  
Vancouver British Columbia V6J 4S6  
Canada

This email is intended for [kristi.saunders@dfo-mpo.gc.ca](mailto:kristi.saunders@dfo-mpo.gc.ca).  
[Update your preferences](#) or [Unsubscribe](#)

delivered by  
 **Campaigner**

## Miller-Saunders, Kristi

---

**Subject:** Update: New Direction on PRV (from ADGT)  
**Location:** DFO CONF Nanaimo-3190HammondBayRd-3-T325 CONF MPO

**Start:** Tue 11/06/2019 3:00 PM  
**End:** Tue 11/06/2019 4:00 PM  
**Show Time As:** Tentative

**Recurrence:** (none)

**Meeting Status:** Not yet responded

**Organizer:** Reid, Farida  
**Required Attendees:** MacDougall, Lesley; Holmes, John; Kennedy, Eddy; Johnson, Stewart; Thiess, Mary; Miller-Saunders, Kristi; Kreiberg, Henrik; Higgins, Mark

Hi everyone,

Lesley would like to meet with you regarding an update on whatever is known about the new direction on PRV.

The meeting is setup for Tuesday June 11 at 3pm for an hour – in the FPP boardroom upstairs.

Thanks,

Farida Reid  
Administrative Assistant, Ecosystems and Oceans Science  
Fisheries and Oceans Canada / Government of Canada  
[farida.reid@dfo-mpo.gc.ca](mailto:farida.reid@dfo-mpo.gc.ca) / Tel: 250-756-7009

Assistante Administrative, Sciences des écosystèmes et des océans  
Pêches et Océans Canada / Gouvernement du Canada  
[farida.reid@dfo-mpo.gc.ca](mailto:farida.reid@dfo-mpo.gc.ca) / Tél: 250-756-7009

## Miller-Saunders, Kristi

---

**From:** Beacham, Terry  
**Sent:** June-07-19 9:22 AM  
**To:** Miller-Saunders, Kristi  
**Subject:** RE: In-season fish condition with Fit-Chips?

What does the Minister's announcement about testing for PRV and HMSI mean for the lab?

---

**From:** Miller-Saunders, Kristi  
**Sent:** Friday, June 07, 2019 9:07 AM  
**To:** Beacham, Terry; Sutherland, Ben  
**Subject:** FW: In-season fish condition with Fit-Chips?

See question about GSI—for Columbia River stock ID in Chinook. Would cost be \$21 per fish? US or Cdn? Also would we use SNPs or microsatellites to distinguish the stocks of interest? How good is our Columbia River baseline?

Potential for new Fit Chip work as well, next year.

Kristi Miller-Saunders, PhD  
Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

**From:** Jennifer L. Gosselin <gosselin@uw.edu>  
**Sent:** June-07-19 8:35 AM  
**To:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Subject:** Re: In-season fish condition with Fit-Chips?

Hi Kristi,

Yes, these samples are originally meant for GSI and I realized after re-reading your abstract that Fit-Chip is for gill tissue. Thanks for the articles too. I am curious how far out one could predict a moribund or poor condition fish (2 days, 2 months, 6 months?), for Snake and Columbia fish passing the hydrosystem. I am looking to see if we can sample next year. Could you please send me the SOP and cost estimate for running samples?

Also, for high throughput GSI, what is the cost for running about 1000 samples? We are looking to identify quite broadly whether fish are from the Snake River basin, Upper Columbia, Middle Columbia or Lower Columbia.

Thank you,  
Jennifer

On Thu, Jun 6, 2019 at 10:19 PM Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca<mailto:Kristi.Saunders@dfo-mpo.gc.ca>> wrote:



I just re-read your email and realized that you have stored your fin clips in ethanol rather than frozen or in RNA-later, and I am afraid that this will not suffice for gene expression studies. That is really too bad as I can certainly see value in the application of the Fit-Chips for your study. Perhaps next year?

I am happy to provide the sampling SOP we use to collect gill tissue for our work, which is usually preserved in RNA-later.

Kristi

---

From: Jennifer L. Gosselin [gosselin@uw.edu<mailto:gosselin@uw.edu>]  
Sent: May 22, 2019 9:21 AM  
To: Miller-Saunders, Kristi  
Subject: Re: In-season fish condition with Fit-Chips?  
Thank you!

On Wed, May 22, 2019 at 9:00 AM Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca<mailto:Kristi.Saunders@dfo-mpo.gc.ca>> wrote:

I will send you back a longer email tomorrow, but here are a few publications either in review or recently out that explain some of the biomarker panels used on the FitChip and infectious agent monitoring work (just sending the Chinook studies).

Houde, ALS, 7 authors, Miller, KM. Discovery and validation of candidate smoltification gene expression biomarkers across multiple species and ecotypes of Pacific salmonids. *Cons Physiol*: In Review. <http://dx.doi.org/10.1101/474692>

Houde, ALS, 6 authors Miller, KM, Salmonid gene expression biomarkers indicative of physiological responses to changes in salinity, temperature, but not dissolved oxygen. *J. Exp. Biol.*: In Review; <http://dx.doi.org/10.1101/491001>.

Akbarzadeh, 6 authors, Miller, KM, 2018. Developing specific molecular biomarkers for thermal stress in salmonids. *BMC genomics*, 19(1): 749. <https://doi.org/10.1186/s12864-018-5108-9>.

Miller, KM, 4 authors. 2017. Molecular indices of viral disease development in wild migrating salmon. *Cons Physiol* 5(1); <https://doi.org/10.1093/conphys/cox036>.

Miller, K.M., 8 authors. 2016. Report on the performance evaluation of the fluidigm biomark<sup>TM</sup> platform for high-throughput microbe monitoring in salmon. CSAS 2014/15 NHQ12

Thakur, KK, 6 authors, KM Miller. 2018. A comparison of infectious agents between hatchery-enhanced and wild out-migrating juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from Cowichan River, British Columbia. *FACETS* 3(1): doi:10.1139/facets-2017-0113

Tucker S, \*Li S, \*Kaukinen KH, Patterson, DA, Miller KM. 2018. Distinct seasonal infectious agent profiles in life-history variants of juvenile Fraser River Chinook salmon; an application of high-throughput genomic screening. *PLOS ONE* 13.4: <https://doi.org/10.1371/journal.pone.0195472>

Jeffries, KM, 5 authors, KM Miller 2014. Immune response genes and pathogen presence predict migration survival in wild salmon smolts. Mol ecol 23: 5803-5815.

More later,

Kristi

---

From: Jennifer L. Gosselin [gosselin@uw.edu<mailto:gosselin@uw.edu>]

Sent: May 21, 2019 4:08 PM

To: Miller-Saunders, Kristi

Subject: Re: In-season fish condition with Fit-Chips?

Kristina,

██████████ I know your name is Kristina. But I must have just typed your email address, and then started the email addressing you by Kristi. ██████████

Jennifer

On Tue, May 21, 2019 at 1:36 PM Jennifer L. Gosselin <gosselin@uw.edu<mailto:gosselin@uw.edu>> wrote:  
Hi Kristi,

You gave a wonderful talk at the NPAFC-IYS workshop. Thank you.

I am very much interested in your Fit-Chip work. I think it lines up closely to some of my work on fish condition of juvenile spring/summer Chinook migrating through the Snake and Columbia hydropower system, and how that can affect survival after hydrosystem passage.

My colleagues and I at the University of Washington and NWFSC/NOAA are currently looking at in-season patterns of smoltification (Na<sup>+</sup>/K<sup>+</sup>-ATPase levels), percent dry mass, fish length, etc. and how those relate to estuary and marine survival. One hypothesis we are interested in is whether highly smolted fish (e.g., collected later in the season and/or further downstream) experience higher survival. This may be because they migrate faster through the estuary and early ocean and are thus less susceptible to predation. Thus far, our preliminary results show that smoltification is an important predictor of survival, and holds more importance in our models than energetic reserves. We are still waiting for one more year of adult returns.

We have fin clips stored in ethanol that we were going to have processed for GSI. But it sounds like Fit-Chip would provide much more information and quite relevant to our main research goal. We have a little over 1000 samples of yearling (spring/summer run) and subyearling (fall run) Chinook salmon samples.

Do you think Fit-Chip would be suitable for our study? Could you please let me know where I can find more information?

Thank you,  
Jennifer

s.19(1)

Jennifer L. Gosselin, Ph.D.  
Research Scientist, Columbia Basin Research School of Aquatic & Fishery Sciences, Univ. of WA  
(206) 685-7316 | gosselin@uw.edu<mailto:gosselin@uw.edu>

NOAA Affiliate, Northwest Fisheries Science Center  
(206) 861-8220 | [jennifer.gosselin@noaa.gov](mailto:jennifer.gosselin@noaa.gov)<mailto:jennifer.gosselin@noaa.gov>

No information has been removed or severed from this page

## Miller-Saunders, Kristi

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**From:** Higgins, Mark  
**Sent:** June-10-19 8:49 AM  
**To:** Miller-Saunders, Kristi; MacDougall, Lesley  
**Cc:** Lowe, Carmel; Moore, Wayne; Parsons, Jay  
**Subject:** RE: summary - today's discussion

Hi Lesley, Yes, thanks for the recap. I have also added a few clarifications into the text in green. Mark.

**From:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Sent:** June-07-19 5:00 PM  
**To:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>; Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>  
**Cc:** Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>; Moore, Wayne <Wayne.Moore@dfo-mpo.gc.ca>; Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>  
**Subject:** RE: summary - today's discussion

Good synopsis Lesley. See my additional comments below

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** June-07-19 3:45 PM  
**To:** Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Cc:** Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>; Moore, Wayne <Wayne.Moore@dfo-mpo.gc.ca>; Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>  
**Subject:** summary - today's discussion

Hi Mark and Kristi;  
First, thank you both for your openness, willingness to work through this and, as always, your expertise.



**Page 767**

**is withheld pursuant to sections  
est retenue en vertu des articles**

**21(1)(b), 21(1)(a)**

**of the Access to Information Act  
de la Loi sur l'accès à l'information**

**AVAILABILITY NEXT WEEK:**

Kristi – will be in Tofino Monday and Tuesday. May be able to join a call on Tuesday

Mark – has the SCC assessment of the Aquatic Animal Health lab against the ISO 12025 standard from Tuesday until Thursday at noon

Lesley – will be in transit Wednesday afternoon, then could participate in a call from Ottawa (assuming someone will vouch for me if I turn up at NHQ), except for a few hours Thursday afternoon.

Lesley MacDougall

A/Division Manager, Aquatic Diagnostics, Genomics & Technology / Division des diagnostics, la génomique, de la technologie aquatique

Fisheries and Oceans Canada / Pêches et Océans Canada

Pacific Biological Station / Station Biologique du Pacifique

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[Lesley.MacDougall@dfo-mpo.gc.ca](mailto:Lesley.MacDougall@dfo-mpo.gc.ca)

s.21(1)(a)

s.21(1)(b)

## Miller-Saunders, Kristi

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**From:** Higgins, Mark  
**Sent:** June-10-19 1:14 PM  
**To:** MacDougall, Lesley; Miller-Saunders, Kristi  
**Cc:** Lowe, Carmel; Moore, Wayne; Parsons, Jay  
**Subject:** RE: summary - today's discussion

I just wanted to clarify as well, from the ministers announcement, it sounded to me that determination of HSMI/Jaundice in aquaculture stocks would be the responsibility of the industry, so DFO may not have any part of this testing, nor input into who would conduct the analysis. For the purposes of the meeting we had on Friday, we talked about 'what if' this portion of the testing came to DFO for analysis, so it was really more a speculative exercise. Mark.

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** June-07-19 3:45 PM  
**To:** Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Cc:** Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>; Moore, Wayne <Wayne.Moore@dfo-mpo.gc.ca>; Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>  
**Subject:** summary - today's discussion

Hi Mark and Kristi;

First, thank you both for your openness, willingness to work through this and, as always, your expertise.

**AVAILABILITY NEXT WEEK:**

Kristi – will be in Tofino Monday and Tuesday. May be able to join a call on Tuesday

Mark – has the SCC assessment of the Aquatic Animal Health lab against the ISO 12025 standard from Tuesday until Thursday at noon

Lesley – will be in transit Wednesday afternoon, then could participate in a call from Ottawa (assuming someone will vouch for me if I turn up at NHQ), except for a few hours Thursday afternoon.

Lesley MacDougall

A/Division Manager, Aquatic Diagnostics, Genomics & Technology / Division des diagnostics, la génomique, de la technologie aquatique

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[Lesley.MacDougall@dfo-mpo.gc.ca](mailto:Lesley.MacDougall@dfo-mpo.gc.ca)

s.21(1)(a)

s.21(1)(b)



## Miller-Saunders, Kristi

---

**From:** Miller-Saunders, Kristi  
**Sent:** June-11-19 2:55 PM  
**To:** Reid, Farida  
**Cc:** MacDougall, Lesley  
**Subject:** RE: Update: New Direction on PRV (from ADGT)

I need a call in number of need Lesley to call me

---

**From:** Reid, Farida  
**Sent:** June 6, 2019 1:54 PM  
**Required:** Reid, Farida; MacDougall, Lesley; Holmes, John; Kennedy, Eddy; Johnson, Stewart; Thiess, Mary; Miller-Saunders, Kristi; Kreiberg, Henrik; Higgins, Mark  
**Subject:** Update: New Direction on PRV (from ADGT)  
**When:** June 11, 2019 3:00 PM-4:00 PM.  
**Where:** DFO CONF Nanaimo-3190HammondBayRd-3-T325 CONF MPO

Hi everyone,

Lesley would like to meet with you regarding an update on whatever is known about the new direction on PRV.

The meeting is setup for Tuesday June 11 at 3pm for an hour – in the FPP boardroom upstairs.

Thanks,

Farida Reid  
Administrative Assistant, Ecosystems and Oceans Science  
Fisheries and Oceans Canada / Government of Canada  
[farida.reid@dfo-mpo.gc.ca](mailto:farida.reid@dfo-mpo.gc.ca) / Tel: 250-756-7009

Assistante Administrative, Sciences des écosystèmes et des océans  
Pêches et Océans Canada / Gouvernement du Canada  
[farida.reid@dfo-mpo.gc.ca](mailto:farida.reid@dfo-mpo.gc.ca) / Tél: 250-756-7009

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** June-17-19 1:54 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re: PSF Board presentation - content needed today if possible

Whatever you have available now (text is fine) and projected for 2019 and 2020. Might also be useful to identify 2-3 key research 'follow up' projects that would be important for PSF to consider (e.g., new viruses, PRV next step, etc). PSF has been provided significant new \$\$ and they will be seeking advice.

[REDACTED]

> On Jun 17, 2019, at 12:11 PM, Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

>

> That was on my list as I need to send in a report to Genome BC as well. But I assume you want something shorter? I have a listing of upcoming publications and ongoing research, with some idea of timelines for getting these works out. How far back to I need to go and what level of detail are you looking for? Something written or a few PPT slides?

>

> Kristi Miller-Saunders, PhD  
> Head, Molecular Genetics  
> Pacific Biological Station  
> 3190 Hammond Bay Rd  
> Nanaimo BC V9T 6N7  
> 250-756-7155  
> Kristi.Saunders@dfo-mpo.gc.ca

>

>

> -----Original Message-----

> From: [REDACTED]  
> Sent: June-17-19 12:09 PM  
> To: Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
> Subject: Fwd: PSF Board presentation - content needed today if possible

>

> Do you have a current summary of progress in SSHI?

>

> [REDACTED]

>

> Begin forwarded message:

>

s.19(1)

> From: [REDACTED]  
> Date: June 17, 2019 at 9:10:15 AM PDT  
> To: [REDACTED]  
> Subject: PSF Board presentation - content needed today if possible

>

> [REDACTED] I am overdue on getting some things together for the Board presentation. One item I need help from you on is an update on SSHI. [REDACTED] has suggested we speak to the following:

>

> · SSHI: Status of analysis and when to expect write up for Board/public?  
> Can you help me with this today? Or is this something I can call Kristi on?

>

> I also need to provide an update on SSMSP. I had a quick call with [REDACTED] this morning and she gave me a verbal update, but if you have anything specific to include let me know. I will send the content I write based on my chat with [REDACTED] over to you later this morning.

>

> Thanks. [REDACTED]

s.19(1)

No further information has been removed or severed from this page

## Miller-Saunders, Kristi

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**From:** Higgins, Mark  
**Sent:** June-17-19 3:00 PM  
**To:** Lowe, Carmel; MacDougall, Lesley; Miller-Saunders, Kristi  
**Cc:** Dickie, Catherine  
**Subject:** RE: Response outstanding: MINO: Key PRV Papers - Timeline for PRV Fish Health Impairment Potential

I have not seen this before. Looks to me like it was developed t [REDACTED] Mark.

**From:** Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>  
**Sent:** June-17-19 2:47 PM  
**To:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>; Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Cc:** Dickie, Catherine <Catherine.Dickie@dfo-mpo.gc.ca>  
**Subject:** FW: Response outstanding: MINO: Key PRV Papers - Timeline for PRV Fish Health Impairment Potential

see request below. I saw this previously attached to some of the info on PRV that was floating about the past couple of months. Nor sure if we generated it or if it came from elsewhere.... Can any of you shed some light on this?

*Carmel*

Carmel Lowe, Ph.D.  
Regional Director Science | Directrice régionale des sciences  
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**From:** Johal, Sharan <Sharan.Jahal@dfo-mpo.gc.ca>  
**Sent:** June 17, 2019 2:08 PM  
**To:** Thomson, Andrew <Andrew.Thomson@dfo-mpo.gc.ca>; Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>  
**Cc:** Barton, Meagan <Meagan.Barton@dfo-mpo.gc.ca>; Dickie, Catherine <Catherine.Dickie@dfo-mpo.gc.ca>; La Chimea, Marisa <Marisa.LaChimea@dfo-mpo.gc.ca>  
**Subject:** Response outstanding: MINO: Key PRV Papers - Timeline for PRV Fish Health Impairment Potential

Hi Andy and Carmel, when you have a moment can you please advise me if you've seen a copy of the attached before? MINO is inquiring and I'd like to respond to them.

Thanks! ☺

s.21(1)(a)  
s.21(1)(b)

Sharan Johal  
Tel : 604-666-1034 / Fax : 604-666-3295 NEW NUMBER  
[sharan.jahal@dfo-mpo.gc.ca](mailto:sharan.jahal@dfo-mpo.gc.ca)

**From:** Johal, Sharan

**Sent:** Tuesday, June 11, 2019 12:41 PM

**To:** Thomson, Andrew <[Andrew.Thomson@dfo-mpo.gc.ca](mailto:Andrew.Thomson@dfo-mpo.gc.ca)>; Webb, Allison <[Allison.Webb@dfo-mpo.gc.ca](mailto:Allison.Webb@dfo-mpo.gc.ca)>; Lowe, Carmel <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>; McCorquodale, Brenda <[Brenda.McCorquodale@dfo-mpo.gc.ca](mailto:Brenda.McCorquodale@dfo-mpo.gc.ca)>

**Cc:** Barton, Meagan <[Meagan.Barton@dfo-mpo.gc.ca](mailto:Meagan.Barton@dfo-mpo.gc.ca)>; Dickie, Catherine <[Catherine.Dickie@dfo-mpo.gc.ca](mailto:Catherine.Dickie@dfo-mpo.gc.ca)>; Delaney, Paula <[Paula.Delaney@dfo-mpo.gc.ca](mailto:Paula.Delaney@dfo-mpo.gc.ca)>

**Subject:** MINO: Key PRV Papers - Timeline for PRV Fish Health Impairment Potential

Good afternoon all, have any of you received the attached before (the Minister has asked).

Thank you

Sharan Johal

A/Team Lead, Executive Secretariat

Regional Director General's Office/Bureau Directeur Général Regional

Fisheries and Oceans Canada - Pacific Region/ Pêches et Océans Canada - Région du Pacifique

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[sharan.johal@dfo-mpo.gc.ca](mailto:sharan.johal@dfo-mpo.gc.ca)

## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** June-18-19 5:28 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** RE: CAHS request for CT values ...

Thanks

**From:** Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]  
**Sent:** June 18, 2019 5:06 PM  
**To:** [REDACTED] Emiliano Di Cicco (emiliano.dicicco@unicam.it) <emiliano.dicicco@unicam.it>  
**Subject:** RE: CAHS request for CT values ...

You were doing fine. I added some content

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**From:** [REDACTED]  
**Sent:** June 17, 2019 11:11 PM  
**To:** Miller-Saunders, Kristi; Emiliano Di Cicco (emiliano.dicicco@unicam.it)  
**Subject:** CAHS request for CT values ...

[REDACTED] understand we have a problem with the PRV test results. I was very glad to hear the analyses were completed but sorry to hear that the CT values were not provided. The importance of the cut-offs relates to interpretation of any differences we observe and the ability of the design to interpret differences. If you recall, I explained the samples had been developed to enter the processing at various stages so that if we did observe differences then we should be able to assist identifying the cause. Part of this analysis was the inclusion of samples with differing levels of PRV load from both fish tissues and artificial construct controls. If only positive/negative results are provided, and not all samples that should test positive are actually scored as positive, it is possible that the sensitivity of your methods may be lower, but without the actual Ct scores, this is harder to establish. Moreover, given the way the test was designed with multiple starting points for many of the same samples, it would be quite easy to evaluate what steps in the process may be causing the problem, if there is one, but again, this is made significantly easier if we can relate the Cts. Your Cts for the same samples with different starting points should be fairly similar, for example. If there is one outlier, that may point us to which aspect of the processing is problematic, again, assuming that any of them are.

So, if you are not willing to send the Ct values because you do not feel that was part of our signed agreement, all you will receive back from us is the proportion of samples correctly identified as detections and no detections.

*Kristi and Emiliano ... while I started to write the email [REDACTED] I realized that I didn't likely understand Kristi's reference to 'controls' during today's discussion. [REDACTED]*

*[REDACTED] I thought that it would be better for one of you to complete this short email about why we need the CT values regardless of the wording in the agreement. Can you do this on Tuesday?? Sorry, but thanks!*

He seems to also miss that if there are differences observed, the interpretation of the results may explain the difference and reduce concerns!! Why wouldn't he provide all information available that may help explain their results?

s.19(1)

[REDACTED] Pacific Salmon Foundation,  
300 – 1682 West 7<sup>th</sup> Avenue, Vancouver, BC

V6J 4S6

604-664-7664 (*office phone*)

604-664-7665 (*office fax*)

 (*cell*)

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## Miller-Saunders, Kristi

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**From:** Parsons, Jay  
**Sent:** June-22-19 6:46 AM  
**To:** [REDACTED]; Olivier, Gilles; 'cstephen@cwhe-rcsf.ca'; Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair; Gagne, Nellie; 'Nathalie.N.Bruneau@inspection.gc.ca'; 'Myron.Roth@gov.bc.ca'; [REDACTED] Miller-Saunders, Kristi; [REDACTED] 'espen.rimstad@nmbu.no'; 'niven@vet.dtu.dk'; 'mark.powell@hi.no'; 'iagardner@upei.ca'; Garver, Kyle; Polinski, Mark; Weber, Lily; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED] 'tony.farrell@ubc.ca'; [REDACTED]; 'Gary.Marty@gov.bc.ca'; [REDACTED]  
Boily, France  
**Subject:** RE: PRV Science Advisory Report now published  
**Attachments:** Table of PRV SAR Comments final.docx

[REDACTED] and all PRV CSAS participants:

In response to [REDACTED] email request (see below) for a summary of participants comments on the CSAS PRV SAR and how these were addressed, please see attached a table of comments reflecting the changes made based on the collective feedback we received.

To summarize the steps taken, following the CSAS meeting, we sought comments on the penultimate version of the SAR from all participants. We received feedback from several participants and the suggestions received were either editorial in nature or more substantive. For the editorial changes, we made those changes where we could, although some comments were dichotomous. As well, there were several substantive comments and these were captured in the attached table of summary of comments. Again where changes could be addressed, the text was revised and where comments could not be addressed, the rationale as to why the changes were not made was noted. All the final changes and rationale were reviewed by the co-chairs, they had additional comments that were addressed and then the final version was approved by the co-chairs as the final arbiters of the SAR.

Best regards,

Jay

**From:** [REDACTED]  
**Sent:** Friday, May 24, 2019 4:08 PM  
**To:** Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>; Olivier, Gilles <Gilles.Olivier@dfo-mpo.gc.ca>; 'cstephen@cwhe-rcsf.ca' <cstephen@cwhe-rcsf.ca>; Burgetz, Ingrid <Ingrid.Burgetz@dfo-mpo.gc.ca>; Waddington, Zac <Zac.Waddington@dfo-mpo.gc.ca>; Struthers, Alistair <Alistair.Struthers@dfo-mpo.gc.ca>; Gagne, Nellie <Nellie.Gagne@dfo-mpo.gc.ca>; 'Nathalie.N.Bruneau@inspection.gc.ca' <Nathalie.N.Bruneau@inspection.gc.ca>; 'Myron.Roth@gov.bc.ca' <Myron.Roth@gov.bc.ca>; [REDACTED] Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; [REDACTED] 'espen.rimstad@nmbu.no' <espen.rimstad@nmbu.no>; 'niven@vet.dtu.dk' <niven@vet.dtu.dk>; 'mark.powell@hi.no' <mark.powell@hi.no>; 'iagardner@upei.ca' <iagardner@upei.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>; Polinski, Mark <Mark.Polinski@dfo-mpo.gc.ca>; Weber, Lily <Lily.Weber@dfo-mpo.gc.ca>; Mimeault, Caroline <Caroline.Mimeault@dfo-mpo.gc.ca>; Holt, Kendra <Kendra.Holt@dfo-mpo.gc.ca>; Johnson, Stewart <Stewart.Johnson@dfo-mpo.gc.ca>; Jones, Simon <Simon.Jones@dfo-mpo.gc.ca>; [REDACTED] 'tony.farrell@ubc.ca' <tony.farrell@ubc.ca>;



'Gary.Marty@gov.bc.ca' <Gary.Marty@gov.bc.ca>;

Boily, France

<France.Boily@dfo-mpo.gc.ca>

**Subject:** Re: PRV Science Advisory Report now published

Thank you Jay:

I have a question though ... rather than have to wade through this document and go by memory on what was written in the previous draft (the one we reviewed), is there any way that you can share:

a) all the reviewers comments on the last draft; and,

b) the draft that was reviewed with track changes so we can see how those comments were addressed and any other changes that might have been made?

I know the draft with track changes may appear messy, but how are we to know if the reviewers' comments were addressed in this final copy?

---

**From:** Parsons, Jay <Jay.Parsons@dfo-mpo.gc.ca>

**Sent:** Friday, May 24, 2019 11:30 AM

**To:** Olivier, Gilles; 'cstephen@cwahc-rcsf.ca'; Burgetz, Ingrid; Waddington, Zac; Struthers, Alistair; Gagne, Nellie; 'Nathalie.N.Bruneau@inspection.gc.ca'; 'Myron.Roth@gov.bc.ca'; [REDACTED] Miller-Saunders, Kristi; [REDACTED] 'espen.rimstad@nmbu.no'; 'niven@vet.dtu.dk'; 'mark.powell@hi.no'; 'iagardner@upei.ca'; Garver, Kyle; Polinski, Mark; Weber, Lily; Mimeault, Caroline; Holt, Kendra; Johnson, Stewart; Jones, Simon; [REDACTED] 'tony.farrell@ubc.ca'; [REDACTED]; 'Gary.Marty@gov.bc.ca'; [REDACTED]; Boily, France

**Subject:** PRV Science Advisory Report now published

Hello CSAS PRV Participants,

I would like, once again, to thank all of you for your participation in the assessment of risk to Fraser River Sockeye Salmon due to piscine orthoreovirus transfer from Atlantic Salmon farms in the Discover Islands area, British Columbia and your input on the final version of the CSAS Science Advisory Report (SAR).

The PRV SAR from the CSAS meeting is now available on the DFO website and the two expected research documents will be following in the upcoming weeks.

Link to publication:

[http://www.dfo-mpo.gc.ca/csas-sccs/Publications/SAR-AS/2019/2019\\_022-eng.html](http://www.dfo-mpo.gc.ca/csas-sccs/Publications/SAR-AS/2019/2019_022-eng.html)

Thank you again for your time throughout this process.

s.19(1)

Jay

**Jay Parsons, PhD**

**Director**

Aquaculture, Biotechnology and Aquatic Animal Health Sciences Branch

Fisheries and Oceans Canada / Government of Canada

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**Directeur**

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### Summary of PRV SAR Comments

Section/Line Number	Original SAR Wording	Comment/Edit Received by Participants	Accepted/Rejected/Modified	Reasoning for Rejection	Suggested Modification
Summary/ Line 26	The Likelihood Assessment concluded that at least one Fraser River Sockeye Salmon, at either the juvenile or adult stage, becoming infected with PRV-1 attributable to Atlantic salmon from in Discovery Islands area is very likely with <u>high uncertainty ranging from</u> <u>high certainty to high uncertainty</u> .	<b>Comment:</b> Someone changed this to a range, but it was agreed upon at the meeting that there was high certainty that at least one sockeye salmon would become infected. There was no contention in the room about this likelihood, and the body of the document supports this.	Modified	The range of uncertainty was added to the summary bullet to be more explicit with what are the range of uncertainties associated with each step of the overall likelihood assessment. We believe the reviewer maybe interpreting the original statement incorrectly.	The overall likelihood assessment concluded that at least one Fraser River Sockeye Salmon, at either the juvenile or adult stage, becoming infected with PRV-1 attributable to Atlantic salmon from in Discovery Islands area is very likely with the uncertainties for the different steps ranged from high certainty to high uncertainty.
Summary/ Lines 30-32	The Consequence Assessment concluded that the potential magnitude <del>of</del> <u>of</u> consequences <del>of</del> to Fraser River Sockeye Salmon abundance and diversity <del>would be</del> <u>is</u> negligible with reasonable certainty for juveniles and reasonable uncertainty for adults. The levels of the uncertainty of this conclusion was discussed and expert participants came to different conclusions on the applicability and abundance of the data to support uncertainty estimates.	<b>Edit:</b> The Consequence Assessment concluded that the potential magnitude of consequences to Fraser River Sockeye Salmon abundance and diversity is negligible. The level of the uncertainty for this conclusion was discussed and expert participants came to different conclusions on the applicability, abundance, and reliability of the data to support uncertainty estimates. A range of uncertainties between reasonable certainty to reasonable uncertainty were associated with this conclusion.	Rejected	This suggested edit was rejected since the proposed wording lumps the potential magnitude of the juveniles and adults together. From discussions at the meeting, it is best to be explicit when referring to juveniles and adults (i.e., separately).	Keep original wording as is in the SAR.
Summary/ Lines 36-39	The main areas where uncertainties were identified were associated with (1) the likelihood of infection because of the lack of data to estimate the	<b>Edit:</b> The main uncertainties were (1) the likelihood of infection of wild salmon PRV-1 from infected Atlantic salmon farms, and (2) the	Modified		The main uncertainties in this risk assessment are:

Section/Line Number	Original SAR Wording	Comment/Edit Received by Participants	Accepted/Rejected/Modified	Reasoning for Rejection	Suggested Modification
	<p>concentration of PRV-1 from infected Atlantic salmon farms, the exposure duration required for infection to occur and the minimum infectious dose for adult and juvenile Sockeye salmon; and (2) the use of proxy data and the laboratory studies provided to estimate consequences to Sockeye Salmon.</p>	<p>consequences to Sockeye Salmon. In the former case, uncertainty exists because of the lack of data to estimate the concentration of PRV-1 from infected Atlantic salmon farms, the exposure duration required for infection to occur and the minimum infectious dose for adult and juvenile Sockeye salmon. For the later, uncertainty exists in applicability laboratory studies to estimate consequences.</p>			<ul style="list-style-type: none"> <li>the high uncertainty related to the likelihood of infection of Fraser River Sockeye Salmon with PRV-1 from infected Atlantic Salmon farms given the lack of data to estimate the concentration of PRV-1 attributable to infected Atlantic Salmon farms, and given that the exposure duration required for infection with PRV-1 to occur and the minimum PRV-1 infectious dose for Sockeye Salmon are unknown; and</li> <li>the reasonable uncertainty related to the consequence assessment given the applicability of proxy data and laboratory studies to estimate consequences for adult Sockeye Salmon.</li> </ul>
<p>Characterization / Lines 57-60</p>	<ul style="list-style-type: none"> <li>PRV-1 infects red blood cells. High loads of PRV-1 have been reported in Atlantic and Sockeye Salmon.</li> <li>In laboratory challenge trials, in juvenile Atlantic or Sockeye Salmon, the high load of PRV-1 is not predictive of disease state.</li> </ul>		Modified		<p>PRV-1 infects red blood cells. In laboratory challenge trials with juvenile Atlantic or Sockeye Salmon, high loads of PRV-1 have been reported. However, it was not predictive of development of disease.</p>

Section/Line Number	Original SAR Wording	Comment/Edit Received by Participants	Accepted/Rejected/Modified	Reasoning for Rejection	Suggested Modification
Characterization / Line 68	PRV-1 has been associated with severe heart inflammation in farmed Atlantic Salmon or jaundice/anemia syndrome in farmed Chinook Salmon in British Columbia; but a causal relationship has not been established.	<b>Edit:</b> In the field, PRV-1 has been statistically associated with severe heart inflammation in farmed Atlantic Salmon and jaundice/anemia syndrome in farmed Chinook Salmon in British Columbia; in both cases, PRV was localized within the regions of tissue damage, but as these were field-based studies, causal relationship was not established.	Modified	In marine net-pens was added for clarity.  Rejected, “in both cases, PRV was localized within the regions of tissue damage, but as these were field-based” as this is too much added detail to the summary bullets agreed upon during the meeting.	In marine net-pens, PRV-1 has been associated with severe heart inflammation in farmed Atlantic Salmon and jaundice/anemia syndrome in farmed Chinook Salmon in British Columbia; but it could not be determined if PRV contributed or not.
Characterization / Lines 70-71	When high viral loads are generated, the BC variant of PRV-1 can cause minor to moderate lesions, but no fish mortalities nor clinical signs nor anaemia were observed.	<b>Edit:</b> “generated, it would appear that the BC variant of PRV-1 may cause minor to moderate heart and/or skeletal muscle inflammation, but no fish mortalities nor anaemia were observed.” [Ed note: This language is more accurate following the publication of Zhang et al. 2019. The authors report that PRV infected fish were diagnosed with heart and/or skeletal muscles inflammation. <b>Comment:</b> This is new information and since this is a draft report, it should more accurately reflect the current state of the science]	Modified	Two studies that demonstrate a statistically significant increase in the prevalence (but not overall severity) of minor heart inflammation in PRV challenged fish relative to controls (Polinski et al 2019; Zhang et al 2019). This was clearly articulated as not being of a severity to warrant a designation of ‘HSMI’ by any current definition of the term in both manuscripts. Skeletal muscle inflammation was not significantly linked with PRV in either of these studies (or any previous study in BC), thus the participants comment is not technically correct.	For clarity.. when high viral loads are generated, the BC variant of PRV-1 <b>enhanced prevalence of</b> minor to moderate <b>heart</b> lesions but no fish mortalities nor clinical signs nor anaemia were observed
Introduction/ Line 90	“This advisory report summarizes the consensus advice developed during the January 28-30,”	<b>Comment:</b> delete the word “consensus” as there was no true consensus	Rejected (but clarification added)	Based on the CSAS definition of consensus.	Please see the SAR for a footnote to the CSAS definition of consensus.

Section/Line Number	Original SAR Wording	Comment/Edit Received by Participants	Accepted/Rejected/Modified	Reasoning for Rejection	Suggested Modification
Characterization / Lines 120-125	PRV-1 predominately infects salmon, although the prevalence of infection in Pacific salmon can vary dramatically between species and stocks, with has been detected more frequently in Coho and Chinook salmon as compared to Chum, Pink, Sockeye salmon and steelhead trout. Sockeye Salmon had lower prevalence and intensity of PRV infections than Atlantic Salmon when cohabitated with PRV-1 positive Atlantic Salmon (Garver et al., 2016a), indicating that Sockeye Salmon are less susceptible to PRV-1 infection than Atlantic Salmon.	<b>Comment:</b> Be very careful in distinguishing between presence and prevalence of the virus and the term "infected". Upon reflection there are no data or reports showing that wild salmon are "infected" with the virus, just that the virus was detected in wild fish populations. Detection does not equate to "infection". Infection is the invasion of an organism's body tissues by disease-causing agents, their multiplication, and the reaction of host tissues to the infectious agents and the toxins they produce.	Rejected	Keep original wording as it is more precautionary.	
Characterization / Lines 131-133	By contrast, in Pacific Canada, PRV-1 has failed to cause severe heart lesions or <u>any severity of skeletal muscle inflammation</u> following experimental challenge of Atlantic or Pacific..	<b>Comment:</b> This is inaccurate. Zhang et al. 2019 report that indeed PRV infected fish exhibited <u>both mild and moderate</u> heart and skeletal muscles lesions. Also, in the draft working papers we reviewed we were advised that these papers, which are now being cited as "published", were "in press". There is a distinct difference in those connotations. Given that these papers are now published the findings therein should be reflected in this document.	Modified	As stated above, the two new studies indicate a significant increase in mild heart inflammation in PRV fish relative to controls (thus implying causation), but not in skeletal muscle inflammation or for severe heart inflammation. The original statement is correct based on these findings. The participant's suggested implication that PRV caused 'both mild and moderate heart and skeletal muscle lesions' is not statistically supported by this data.	Suggest removing "any severity" (still keeps integrity of the sentence).  By contrast, in Pacific Canada, PRV-1 has failed to cause severe heart lesions or <u>skeletal muscle inflammation</u> following experimental challenge of Atlantic or Pacific..  Modified to: "By contrast, in Pacific Canada, BC PRV-1 has failed to cause severe heart lesions or skeletal muscle inflammation following experimental challenge of Atlantic or Pacific salmon (Garver et al.,

Section/Line Number	Original SAR Wording	Comment/Edit Received by Participants	Accepted/ Rejected/ Modified	Reasoning for Rejection	Suggested Modification
Characterization /Line 142	In Pacific Canada, although PRV-1 is highly prevalent in farmed Atlantic Salmon and two subclinical farm-level cases of HSMI-like disease have been suggested to date (Di Cicco et al., 2017; Polinski et al., 2019), clinical outbreak of HSMI as described in Norway has never been reported.	<b>Edit:</b> Pacific Canada, although PRV-1 is highly prevalent in farmed Atlantic Salmon and two farm-level cases of HSMI disease have been suggested to date (Di Cicco et al., 2017; Polinski et al., 2019), clinical outbreaks of HSMI causing moderate levels mortality, described in Norway, have never been reported. <b>Comment:</b> The case of HSMI described in Di Cicco et al. demonstrated both gross and pathological signs of the disease, and low level mortality in only a few net pens. It was not subclinical—as the vet on the farm sent samples for pathological investigation at the peak of the disease due to elevated mortalities and behavioural changes in the fish—it did not go unnoticed, as would be expected if it were truly subclinical.	Modified	The participants comments are correct in that there was low level mortality on the farm that was sampled during Di Cicco et al. 2017. Whether PRV had anything to do with the mortality is guesswork. Can either suggest accepting the participant's edit, or alter the statement to reflect clinical case definitions of HSMI in Norway that deal with more than just mortality.	2016a; Polinski et al., 2019; Zhan et al., 2019)."  ...HSMI-like disease have been suggested to date (Di Cicco et al. 2017; Polinski et al., 2019), clinic presentation of disease as originally used in the case definition of HSMI as it occurs in Norway (Kongtorp et al 2004a; 2004b) has never been reported in Pacific Canada.  Kongtorp, R., Taksdal, T. & Lyngø A. Pathology of heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon <i>Salmo salar</i> . Diseases of Aquatic Organisms 59, 217–224 (2004).  Kongtorp, R. T., Kjerstad, A., Taksdal, T., Guttvik, A. & Falk, K. Heart and skeletal muscle inflammation in Atlantic salmon, <i>Salmo salar</i> L.: a new infectious disease. Journal of Fish Diseases 27, 351–358 (2004).
Characterization / Lines 159-164	Sockeye Salmon injected with PRV developed considerable blood and kidney PRV loads but no weight loss, morbidity or pathology could be attributed to the virus (Polinski et al., 2016). Despite high prevalence and persistence of PRV in blood and kidney	<b>Edit:</b> Sockeye Salmon injected with PRV developed considerable blood and kidney PRV loads but no weight loss or morbidity could be attributed to the virus, and pathology was inconclusive (Polinski et al., 2016). Despite high	Rejected	Do not agree that the pathology was inconclusive; the pathology was indeed conclusive for the presence or absence of significant disease, there was just one instance where significant disease was	Keep original wording.

Section/Line Number	Original SAR Wording	Comment/Edit Received by Participants	Accepted/Rejected/Modified	Reasoning for Rejection	Suggested Modification
	<p>of Sockeye Salmon cohabitated with PRV positive Atlantic Salmon, no microscopic lesions, disease or mortality could be attributed to the virus (Garver et al., 2016a). Preliminary data indicate that PRV infections are inconsequential to Sockeye Salmon respiratory function (see Polinski and Garver, in review).</p>	<p>prevalence and persistence of PRV in blood and kidney of Sockeye Salmon cohabitated with PRV positive Atlantic Salmon, no disease or mortality could be attributed to the virus, but again, pathology was inconclusive (Garver et al., 2016a). Preliminary data indicate that PRV infections in the absence of disease are inconsequential to Sockeye Salmon respiratory function (see Polinski and Garver, in review). To date, there is no evidence that PRV causes disease in Sockeye Salmon despite successful infection with the virus under experimental conditions (Garver et al., 2016a; Garver et al., 2016b; Polinski et al., 2016).</p> <p><b>Comment:</b> As pointed out in the meeting, not a single one of these studies on sockeye salmon took sufficient tissues for histopathological evaluation to statistically determine if there were lesions of significance. It is entirely untrue to say that there were "No microscopic lesions", and there was no way to attribute what was observed to the virus due to lack of controls. Throughout this review, conclusions based on pathology should be clarified as being inconclusive. Even Gary</p>		<p>observed and we cannot say one way or another if PRV was or was not involved.</p> <p>In context, there are three published studies and one manuscript in preparation that challenged Sockeye Salmon with PRV. In each instance, the primary objective was to see if any of the challenged fish developed a disease state and therefore board certified veterinary pathologists were asked if they thought significant disease (i.e., enough damage to compromise organ or organism function) was present. This is a dichotomous diagnostic outcome (tissues were either significantly diseased or they were not) and just like any other diagnostic dichotomous test, time matched controls are irrelevant to the outcome unless you are trying to prove causation following a positive detection. The outcomes are as follows:</p> <p><u>Garver et al 2016b</u>  -20 wpc; liver and hearts; n=10  – <b>no significant disease</b> (time-matched controls also considered)</p>	



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		Marty agreed to this. Moreover, in one of their studies, there were actually mortalities with fish dying of lesions that were not inconsistent with the anticipated disease (as mentioned by Dr. Marty in the meeting), but these were ignored in the paper, again due to lack of controls.		Polinski et al 2016 -3 wpc; kidney and skeletal muscle; n=8 – <b>no significant disease</b> (time-matched controls also considered) -15 wpc; kidney and skeletal muscle; n=8 – <b>no significant disease</b> (time-matched controls also considered) Garver et al 2016a -2-6 wpc; gill, skeletal muscle, eye, heart, spleen, liver, kidney, pyloric caeca, brain and intestine; n=6 (3@2wpc, 1@4wpc, 2@6wpc) – <b>no significant disease</b> (fish were still PRV negative at this time).	
Consequence Assessment/ Line 316	Assuming that results from laboratory studies on the impact of PRV-1 infection in juvenile Sockeye Salmon are indicative of what occurs in the marine environment, it was concluded with reasonable certainty that the potential magnitude of consequences to Fraser River Sockeye Salmon abundance and diversity would be negligible.	I believe that the conclusion for juvenile and adult salmon ranged from reasonable uncertainty to reasonable certainty.	Modified	To keep the original uncertainty stated (reasonable certainty) as the majority agreed on reasonable uncertainty.	A footnote will be added to make reference to the uncertainties in the consequence assessment section to highlight the other uncertainties expressed.
Consequence Assessment/ Line 321	To date, the only study reporting on the impacts of PRV-1 infection in adult Sockeye Salmon indicated that infection with PRV-1 in the marine	<b>Edit:</b> To date, the only study reporting on the impacts of PRV-1 infection in adult Sockeye Salmon indicated that while infection with PRV-1 in the marine environment	Modified		To date, the only study reporting on the impacts of PRV-1 infection in adult Sockeye Salmon looked in two Fraser River Sockeye Salmon stocks. While infection with PRV-

Section/Line Number	Original SAR Wording	Comment/Edit Received by Participants	Accepted/Rejected/Modified	Reasoning for Rejection	Suggested Modification
	environment did not significantly affect the odds of dying before reaching spawning grounds in two Fraser River Sockeye Salmon stocks (Miller et al., 2014).	was associated with migratory losses as fish entered the river for one stock, the odds ratio of mortality to spawning grounds between infected and uninfected individuals was not significant (Miller et al., 2014).			in the marine environment was associated with migratory losses as fish entered the river for one stock, the opposite was reported in the other stock. Additionally, the odds ratio of mortality to spawning grounds between infected and uninfected individuals was not significant in both stocks (Miller et al., 2014).
Consequence Assessment/ Line 348	...the potential magnitude of consequences to Fraser River Sockeye Salmon abundance and diversity would be negligible	Change "would be negligible" to "is likely negligible."	Rejected	Changing the wording from "would be" to "likely be" changes the meaning of what was agreed upon during the CSAS meeting ; abundance and diversity would be negligible.	
Conclusions/ Lines 441-443	The levels of the uncertainty attributed to the magnitude of consequences ranged from reasonable certainty to reasonable uncertainty. Expert participants came to different conclusions on the applicability and abundance of the data to support uncertainty estimates.	<b>Comment:</b> To move the sentences pertaining to the uncertainties near the beginning of the paragraph. This provides one paragraph conclusion on consequence and combines with uncertainty.	Accepted		Moved the sentences closer to the stated level of risk.

## Miller-Saunders, Kristi

---

**From:** Amy Teffer <[REDACTED]>  
**Sent:** June-24-19 5:28 PM  
**To:** Miller-Saunders, Kristi  
**Cc:** Jon Carr; Schulze, Angela; Bradbury, Ian R; Tabata, Amy; denise.deschamps@mffp.gouv.qc.ca; [REDACTED] carole-anne gillis; Gideon Mordecai  
**Subject:** Atlantic salmon infectious agent screening manuscript  
**Attachments:** Manuscript Draft\_Atl salmon infection assessment\_6-24-2019.docx

Good evening co-authors,

We have a final draft for your review prior to journal submission (Facets). I'm pleased with how this paper has turned out. A great deal of time and effort went into data collection, analysis, and write up - thanks everyone! (And please don't forget to thank anyone who is not an author within the Acknowledgments section).

I would appreciate any comments or edits (tracked changes or a bulleted list) returned to me by Friday of next week (July 5) so that I can submit the final manuscript as soon as possible. Please make sure that your affiliations are correct and acknowledgements/relevant funding sources are added.

There are only 3 remaining details that I need relevant to data collection and sample processing (lengths of 2016 Greenland fish, days at -18C for Greenland samples, GenBank IDs for viral sequences). Other than that, the manuscript is complete.

Feel free to reach out to me with any questions or concerns. Thanks again and I hope everyone is enjoying their summer.

Best,  
Amy

--

><=> ><=> ><=>

Amy K. Teffer, PhD



s.19(1)

**Pages 790 to / à 842**  
**are withheld pursuant to sections**  
**sont retenues en vertu des articles**

**21(1)(b), 13(1)(c), 21(1)(a)**

**of the Access to Information Act**  
**de la Loi sur l'accès à l'information**

## Miller-Saunders, Kristi

---

**From:** Higgins, Mark  
**Sent:** June-26-19 10:30 AM  
**To:** Webb, Allison; MacDougall, Lesley; Miller-Saunders, Kristi  
**Subject:** RE: PRV costing

Allison, can I assume that AMD will be performing necropsies in the field (your cost) such that you will be able to collect tissues for broader audit purposes if you so desire? If necropsies are done in our lab, I would need to know what tissues you would like collected and preservation types, etc.

I will also provide a draft look at how we may wish to approach SEP sampling in the spring, knowing that this is really just in preliminary planning with little information available.

Mark.

**From:** Webb, Allison <Allison.Webb@dfo-mpo.gc.ca>  
**Sent:** June-26-19 10:22 AM  
**To:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>; Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Subject:** Re: PRV costing

I know. Just send me anything that have and I will consolidate it and send up later this aft. I won't be working on it for a few hours so you have some time. Thanks!

Sent from my BlackBerry 10 smartphone on the Bell network.

**From:** MacDougall, Lesley  
**Sent:** Wednesday, June 26, 2019 1:13 PM  
**To:** Higgins, Mark; Miller-Saunders, Kristi  
**Cc:** Webb, Allison  
**Subject:** RE: PRV costing

Damn. I had hoped we would be able to have the scoping meeting before we were required to provide costing, but it looks like something at least is required by **today**.

So – at this point we had identified 2 EG03 terms,  
And had identified a cost of \$40 - \$60 per fish, but that didn't include the genomic analysis time? Is that correct?  
And this is contingent on Science receiving tissue samples.

Are there other costs for consumables, for analysis, other personnel, equipment maintenance, etc?

s.21(1)(a)  
s.21(1)(b)

Lesley

**From:** Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>  
**Sent:** Wednesday, June 26, 2019 10:09 AM  
**To:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>; Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>

**Subject:** FW: PRV costing

**Importance:** High

Yikes – looks like we will need to come up with a first order estimate of the costs for testing – even in the absence of the details..... as only source of funds is the carry forward from last year.

Lesley – can you work with Mark and Kristie to develop something today?

*Carmel*

Carmel Lowe, Ph.D.  
Regional Director Science | Directrice régionale des sciences  
Fisheries and Oceans Canada | Pêches et Océans Canada  
Pacific Biological Station | Station biologique du Pacifique  
3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7  
[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)  
Telephone | Téléphone 250-756-7177  
Facsimile | Télécopieur 250-729-8360  
Government of Canada | Gouvernement du Canada

**From:** Thomson, Andrew <[Andrew.Thomson@dfo-mpo.gc.ca](mailto:Andrew.Thomson@dfo-mpo.gc.ca)>

**Sent:** June 26, 2019 9:56 AM

**To:** Webb, Allison <[Allison.Webb@dfo-mpo.gc.ca](mailto:Allison.Webb@dfo-mpo.gc.ca)>; Webb, Cheryl <[Cheryl.Webb@dfo-mpo.gc.ca](mailto:Cheryl.Webb@dfo-mpo.gc.ca)>; Lowe, Carmel <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>

**Cc:** To, Loretta <[Loretta.To@dfo-mpo.gc.ca](mailto:Loretta.To@dfo-mpo.gc.ca)>

**Subject:** PRV costing

**Importance:** High

Need a defensible \$ value for PRV testing requirements this year so that we can seek funds from the carry over, and we may need today.

We can break it up by sector FM/SEP/Science or combine just so long as were clear about the ask.

Andrew J L Thomson

Regional Director | Directeur régional  
Fisheries Management Branch | Direction de la gestion des pêches  
Pacific Region | Région du Pacifique  
Fisheries & Oceans Canada | Pêches et Océans Canada  
Suite 200 – 401 Burrard St.  
Vancouver, BC, Canada V6C 3S4  
[andrew.thomson@dfo-mpo.gc.ca](mailto:andrew.thomson@dfo-mpo.gc.ca)  
Telephone | Téléphone 604.666.0751  
Facsimile | Télécopieur 250.666.8069  
Government of Canada | Gouvernement du Canada

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** June-26-19 12:11 PM  
**To:** MacDougall, Lesley; Higgins, Mark  
**Cc:** Webb, Allison  
**Subject:** RE: PRV costing

Working on it

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** June-26-19 10:14 AM  
**To:** Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Cc:** Webb, Allison <Allison.Webb@dfo-mpo.gc.ca>  
**Subject:** RE: PRV costing

Damn. I had hoped we would be able to have the scoping meeting before we were required to provide costing, but it looks like something at least is required by **today**.

So – at this point we had identified 2 EG03 terms,  
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And this is contingent on Science receiving tissue samples.

Are there other costs for consumables, for analysis, other personnel, equipment maintenance, etc?

Lesley

**From:** Lowe, Carmel <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>  
**Sent:** Wednesday, June 26, 2019 10:09 AM  
**To:** MacDougall, Lesley <[Lesley.MacDougall@dfo-mpo.gc.ca](mailto:Lesley.MacDougall@dfo-mpo.gc.ca)>; Higgins, Mark <[Mark.Higgins@dfo-mpo.gc.ca](mailto:Mark.Higgins@dfo-mpo.gc.ca)>; Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>  
**Subject:** FW: PRV costing  
**Importance:** High

Yikes – looks like we will need to come up with a first order estimate of the costs for testing – even in the absence of the details..... as only source of funds is the carry forward from last year.

Lesley – can you work with Mark and Kristie to develop something today?

*Carmel*

Carmel Lowe, Ph.D.  
Regional Director Science | Directrice régionale des sciences  
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3190 Hammond Bay Rd, Nanaimo, BC, Canada V9T 6N7

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Facsimile | Télécopieur 250-729-8360  
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**From:** Thomson, Andrew <[Andrew.Thomson@dfo-mpo.gc.ca](mailto:Andrew.Thomson@dfo-mpo.gc.ca)>  
**Sent:** June 26, 2019 9:56 AM  
**To:** Webb, Allison <[Allison.Webb@dfo-mpo.gc.ca](mailto:Allison.Webb@dfo-mpo.gc.ca)>; Webb, Cheryl <[Cheryl.Webb@dfo-mpo.gc.ca](mailto:Cheryl.Webb@dfo-mpo.gc.ca)>; Lowe, Carmel <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>  
**Cc:** To, Loretta <[Loretta.To@dfo-mpo.gc.ca](mailto:Loretta.To@dfo-mpo.gc.ca)>  
**Subject:** PRV costing  
**Importance:** High

Need a defensible \$ value for PRV testing requirements this year so that we can seek funds from the carry over, and we may need today.

We can break it up by sector FM/SEP/Science or combine just so long as were clear about the ask.

Andrew J L Thomson

Regional Director | Directeur régional  
Fisheries Management Branch | Direction de la gestion des pêches  
Pacific Region | Région du Pacifique  
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[andrew.thomson@dfo-mpo.gc.ca](mailto:andrew.thomson@dfo-mpo.gc.ca)  
Telephone | Téléphone 604.666.0751  
Facsimile | Télécopieur 250.666.8069  
Government of Canada | Gouvernement du Canada



## Miller-Saunders, Kristi

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**From:** Higgins, Mark  
**Sent:** June-26-19 4:04 PM  
**To:** MacDougall, Lesley; Miller-Saunders, Kristi  
**Subject:** RE: PRV costing  
**Attachments:** [REDACTED] DFO AAHL Cost Estimate for testing for PRV survey.docx

Here is my first draft on costing. [REDACTED]  
[REDACTED]  
[REDACTED]

**From:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Sent:** June-26-19 10:14 AM  
**To:** Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Cc:** Webb, Allison <Allison.Webb@dfo-mpo.gc.ca>  
**Subject:** RE: PRV costing

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And had identified a cost of \$40 - \$60 per fish, but that didn't include the genomic analysis time? Is that correct?  
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Lesley

**From:** Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>  
**Sent:** Wednesday, June 26, 2019 10:09 AM  
**To:** MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>; Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>  
**Subject:** FW: PRV costing  
**Importance:** High

Yikes – looks like we will need to come up with a first order estimate of the costs for testing – even in the absence of the details..... as only source of funds is the carry forward from last year.

Lesley – can you work with Mark and Kristie to develop something today?

*Carmel*

s.21(1)(a)  
s.21(1)(b)  
s.23

Carmel Lowe, Ph.D.  
Regional Director Science | Directrice régionale des sciences  
Fisheries and Oceans Canada | Pêches et Océans Canada  
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Carmel.Lowe@dfo-mpo.gc.ca

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Government of Canada | Gouvernement du Canada

**From:** Thomson, Andrew <[Andrew.Thomson@dfo-mpo.gc.ca](mailto:Andrew.Thomson@dfo-mpo.gc.ca)>

**Sent:** June 26, 2019 9:56 AM

**To:** Webb, Allison <[Allison.Webb@dfo-mpo.gc.ca](mailto:Allison.Webb@dfo-mpo.gc.ca)>; Webb, Cheryl <[Cheryl.Webb@dfo-mpo.gc.ca](mailto:Cheryl.Webb@dfo-mpo.gc.ca)>; Lowe, Carmel <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>

**Cc:** To, Loretta <[Loretta.To@dfo-mpo.gc.ca](mailto:Loretta.To@dfo-mpo.gc.ca)>

**Subject:** PRV costing

**Importance:** High

Need a defensible \$ value for PRV testing requirements this year so that we can seek funds from the carry over, and we may need today.

We can break it up by sector FM/SEP/Science or combine just so long as were clear about the ask.

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Fisheries Management Branch | Direction de la gestion des pêches

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**Pages 849 to / à 852  
are withheld pursuant to section  
sont retenues en vertu de l'article**

**23**

**of the Access to Information Act  
de la Loi sur l'accès à l'information**

## Miller-Saunders, Kristi

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**From:** Miller-Saunders, Kristi  
**Sent:** June-26-19 4:47 PM  
**To:** MacDougall, Lesley  
**Subject:** costing PRV sequencing

It is possible that if the number of detections for sequencing was considerably lower, or if testing [REDACTED]  
[REDACTED] However, I see this as an opportunity to stabilize funding, at least for the short term, of  
one of my technical staff. I do not have an EG04, nor do I think the skill set to do the phylogenetics work is at a level 04.

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
Kristi.Saunders@dfo-mpo.gc.ca

s.21(1)(a)

s.21(1)(b)

## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** June-26-19 8:12 PM  
**To:** Garver, Kyle; Polinski, Mark  
**Cc:** [REDACTED] McCorquodale, Brenda; Paylor, Adrienne; DiCicco, Emiliano; Miller-Saunders, Kristi; MacDougall, Lesley; Shaw, Kerra; Mollins, Jennifer; Mark.Higgins@dfo.mpo.gc.ca; Jones, Simon; Taekema, Bernie John; Manchester, Howie  
**Subject:** Email  
**Attachments:** A-2016-01101 Rimstad redacted results.pdf

Hello All

Thank you for the good meeting today. Perhaps you could forward this to your new epidemiologist, whose name I can't find on the list of participants.

I am attaching the email that I mentioned wherein Espen Rimstad reports on the first trial with PRV from BC in Atlantic salmon. It sounds like he is saying that that PRV from BC produced lesions in Atlantic salmon in Norway that were graded by his histopathologist.

However, the work presented today by Mark Polinski reports the opposite.

In the spirit of transparency, could we see the unredacted version of this email?

Thanks so much,



s.19(1)

## Garver, Kyle

---

**From:** Espen Rimstad <espen.rimstad@nmbu.no>  
**Sent:** April-05-16 10:04 AM  
**To:** Garver, Kyle  
**Subject:** SV: skype call

Fine, then we settle for April 12th.  
Espen

---

**Fra:** Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>  
**Sendt:** 5. april 2016 00:43:19  
**Til:** Espen Rimstad  
**Kopi:** Øystein Wessel  
**Emne:** RE: skype call

Hi Espen,

Very exciting to hear of the BC challenge. We (Mark and I) could skype with you April 12<sup>th</sup> or 13<sup>th</sup> at 7AM Pacific time (4 PM Oslo time). Alternatively we could call during our evening time (10 PM Pacific) which would be 7 AM your time.

Looking forward to speaking with you soon.  
Kyle

s.20(1)(b)

s.21(1)(a)

s.21(1)(b)

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**From:** Espen Rimstad [mailto:espen.rimstad@nmbu.no]  
**Sent:** April-04-16 8:45 AM  
**To:** Garver, Kyle  
**Cc:** Øystein Wessel  
**Subject:** SV: skype call

Hi Kyle,

Last Friday we had a Skype meeting with Emiliano et co. Are you aware of their findings? Østein and myself feel a bit in a Catch 22 position.

But we would like to inform you about the first outcome of the experimental challenge with the BC isolate you send us. We will organise a file with details later.

The histopat is done by someone outside our department, and they grade it for us (1-3). I would not recommend to use energy about the question if PRV causes HSMI or not (i.e. if you need additional factors or not) we have firm evidence that this is not necessary.

000039

000855

Regards,  
Espen

---

**Fra:** Garver, Kyle <[Kyle.Garver@dfo-mpo.gc.ca](mailto:Kyle.Garver@dfo-mpo.gc.ca)>

**Sendt:** 29. mars 2016 12:55

**Til:** Espen Rimstad

**Emne:** Out of Office AutoReply: skype call

I'm out of the office until April 4, 2016 and have limited access to email.

Regards,

Kyle

Kyle Garver, Ph.D.

Fisheries and Oceans Canada

Pacific Biological Station

Aquatic Animal Health

3190 Hammond Bay Road Nanaimo, BC V9T 6N7

Phone:(250)756-7340

Fax: (250) 756-7053

email: [Kyle.Garver@dfo-mpo.gc.ca](mailto:Kyle.Garver@dfo-mpo.gc.ca)

## Miller-Saunders, Kristi

---

**From:** Higgins, Mark  
**Sent:** June-27-19 9:12 AM  
**To:** Choi, Shirley; Lowe, Carmel; Webb, Allison; MacDougall, Lesley  
**Cc:** Miller-Saunders, Kristi  
**Subject:** Re: DFO Science - cost estimate for PRV screening and sequencing tests

Thank you Shirley. Mark.

Sent from my Bell Samsung device over Canada's largest network.

----- Original message -----

**From:** "Choi, Shirley" <Shirley.Choi@dfo-mpo.gc.ca>  
**Date:** 2019-06-27 9:03 AM (GMT-08:00)  
**To:** "Lowe, Carmel" <Carmel.Lowe@dfo-mpo.gc.ca>, "Higgins, Mark" <Mark.Higgins@dfo-mpo.gc.ca>, "Webb, Allison" <Allison.Webb@dfo-mpo.gc.ca>, "MacDougall, Lesley" <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Cc:** "Miller-Saunders, Kristi" <Kristi.Saunders@dfo-mpo.gc.ca>  
**Subject:** RE: DFO Science - cost estimate for PRV screening and sequencing tests

No problem! On my spreadsheet, I am differentiating costs as either O&M and S&W. I will note somewhere that if only O&M is provided, they will need to increase it by the conversion rate.

Shirley

**From:** Lowe, Carmel <Carmel.Lowe@dfo-mpo.gc.ca>  
**Sent:** Thursday, June 27, 2019 8:41 AM  
**To:** Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>; Webb, Allison <Allison.Webb@dfo-mpo.gc.ca>; MacDougall, Lesley <Lesley.MacDougall@dfo-mpo.gc.ca>  
**Cc:** Miller-Saunders, Kristi <Kristi.Saunders@dfo-mpo.gc.ca>; Choi, Shirley <Shirley.Choi@dfo-mpo.gc.ca>  
**Subject:** Re: DFO Science - cost estimate for PRV screening and sequencing tests

Good point Mark - definitely should include these costs.

Sent from my Bell Samsung device over Canada's largest network.

----- Original message -----

**From:** "Higgins, Mark" <Mark.Higgins@dfo-mpo.gc.ca>  
**Date:** 2019-06-27 7:49 AM (GMT-08:00)  
**To:** "Webb, Allison" <Allison.Webb@dfo-mpo.gc.ca>, "MacDougall, Lesley" <Lesley.MacDougall@dfo-mpo.gc.ca>



Cc: "Miller-Saunders, Kristi" <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>, "Lowe, Carmel" <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>, "Choi, Shirley" <[Shirley.Choi@dfo-mpo.gc.ca](mailto:Shirley.Choi@dfo-mpo.gc.ca)>  
Subject: Re: DFO Science - cost estimate for PRV screening and sequencing tests

Looks good to me Lesley, one point that I noticed is that we have put in for salary \$\$, but if we get a lump sum transfer, o&m will have to be converted at x1.27. Not sure if this should be reflected in our ask or not Mark.

Sent from my Bell Samsung device over Canada's largest network.

----- Original message -----

From: "Webb, Allison" <[Allison.Webb@dfo-mpo.gc.ca](mailto:Allison.Webb@dfo-mpo.gc.ca)>  
Date: 2019-06-26 6:33 PM (GMT-08:00)  
To: "MacDougall, Lesley" <[Lesley.MacDougall@dfo-mpo.gc.ca](mailto:Lesley.MacDougall@dfo-mpo.gc.ca)>  
Cc: "Higgins, Mark" <[Mark.Higgins@dfo-mpo.gc.ca](mailto:Mark.Higgins@dfo-mpo.gc.ca)>, "Miller-Saunders, Kristi" <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>, "Lowe, Carmel" <[Carmel.Lowe@dfo-mpo.gc.ca](mailto:Carmel.Lowe@dfo-mpo.gc.ca)>, "Choi, Shirley" <[Shirley.Choi@dfo-mpo.gc.ca](mailto:Shirley.Choi@dfo-mpo.gc.ca)>  
Subject: RE: DFO Science - cost estimate for PRV screening and sequencing tests

Thanks so much for this Lesley. I just finished talking to Shirley Choi who is going to put all of this together for us. I just reviewed the framework of the excel tables with her. She will pop this into it and if she has any questions, she'll give you a call tomorrow.

This is due at 2pm so there might even be a chance that Shirley can finish this in the am and send around to everyone for a quick check before sending it to Andy in the afternoon.

I really appreciate everyone's help. I'll be in transit more of tomorrow, but will check first thing in the am and when I transfer flights.

Thanks again,  
Allison

---

**From:** MacDougall, Lesley  
**Sent:** June 26, 2019 6:28 PM  
**To:** Webb, Allison  
**Cc:** Higgins, Mark; Miller-Saunders, Kristi; Lowe, Carmel  
**Subject:** DFO Science - cost estimate for PRV screening and sequencing tests

Hi Allison;  
Attached is a draft of the cost breakdown for both the Aquatic Animal Health and the Molecular Genetics Laboratories, based potential testing scenarios [REDACTED]

As I amalgamated input from both Mark and Kristi I've asked them to give it one last look to ensure I didn't mess something up; if there are any corrections I'll get them to you tomorrow.  
Also, I've attached an email (referred to in the costing document) regarding the patent that is used for the diagnostic testing. [REDACTED]  
[REDACTED]

If you have any questions please give me a call or shoot an email over, I'm in all day tomorrow [REDACTED]  
[REDACTED]

s.19(1)  
s.21(1)(a)  
s.21(1)(b)  
s.23

Lesley MacDougall

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Fisheries and Oceans Canada / Pêches et Océans Canada

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250-756-7395

[Lesley.MacDougall@dfo-mpo.gc.ca](mailto:Lesley.MacDougall@dfo-mpo.gc.ca)

Lesley

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## Miller-Saunders, Kristi

---

**From:** [REDACTED]  
**Sent:** June-27-19 11:00 AM  
**To:** Minister / Ministre (DFO/MPO)  
**Cc:** [REDACTED] McCorquodale, Brenda; [REDACTED] Garver, Kyle; Polinski, Mark; [REDACTED] Miller-Saunders, Kristi; DiCicco, Emiliano; [REDACTED]  
**Subject:** Meeting with AMD - concerns  
**Attachments:** A-2016-01101 Rimstad redacted results.pdf

Dear Minister Wilkinson

I met with staff and scientists in your Aquaculture Management Division (AMD) yesterday as part of ongoing meetings organized by the Conservation Regulatory Working Group of which I am a member. I am grateful your staff took the time to meet with us.

I was, of course, disappointed that the technical working groups that you announced on June 4th won't be formed in time to inform your impending decision on your PRV Policy.

**I am concerned that your PRV screening has not begun as per your announcement a month ago** as transfers are underway. I am also concerned that unprecedented decisions are being made in-house about the relative virulence of PRV isolates and thus which PRV variants you will be permitting in farm salmon transfers. These decision are being made in absence of information on the impact of this virus on wild salmon species in their natural environment. What information we do have triggers the Precautionary Principle in particular the recent research on impact on juvenile Chinook salmon migrating past salmon farms.

**PRV is an internationally recognized disease agent**, and as such it is captured by section 56(b) of the FGR. To allow some PRV isolates to enter fish farms, where amplification and thus mutation will occur appears reckless given the critical state of wild salmon in BC. I realize you are proposing to alter S. 56(b) to all all strains of PRV in transfers, and I will be addressing that separately, but what is apparent is the over-arching willingness by DFO to permit the spread of PRV to wild fish in support of the salmon farming industry. This is both a threat to wild salmon and the salmon farming industry's rapidly receding social licence.

**Drs. Garver and Mark Polinski reported on recent research yesterday.** It was good to see that their research is now producing results that are highly comparable to research I published in 2017, as well as work by others who are also reporting that salmon become infected with PRV when exposed to salmon farms that are known to be infected with PRV. It is concerning that they highlighted that Chinook and Coho appear to be at the greatest risk, something we also see in the research by Di Cicco and Wang.

**Specifically, Polinski reported that when PRV-free Cermaq farm salmon are placed in farms near Marine Harvest in the Broughton and Discovery Islands or near Creative Salmon farms in Clayoquot Sound, they become infected with PRV in 100 days, but when placed in Departure Bay near PBS where there are no farms, they do not become infected.** Marine Harvest swore an affidavit stating that all but one of their hatcheries is PRV-positive and of course Creative Salmon livestock suffer from jaundice related to PRV and recent footage reveals those jaundice salmon are still in the Creative farms (see picture).

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**Dr. Garver reported that PRV work in a Norwegian lab** suggests lower virulence in a BC isolate of PRV. In the interest of transparency I have requested an unredacted version of the attached email wherein this Norwegian lab is reporting on initial tests. This email is so critical because it also recommends that DFO stop questioning whether a secondary factor is required to cause disease in PRV-infected fish. If Dr. Rimstad's email reports that the BC isolate of PRV provided to his lab in 2016 by Dr. Garver produced HSMI lesions, as it appears, we need to know more about this experiment and the current results reported by Garver.

**Currently, it is my view that the Aquaculture Management Division is making decisions** based on the economic needs of the salmon farming industry which are placing wild salmon at increasing risk. Marine Harvest told the court they would be "severely impacted" if prohibited from transferring PRV-infected fish from their hatcheries into marine farms, which have been sited in the most important wild fish habitat in BC. AMD research on PRV has reported outlier conclusions, but now that Polinski's results report 100% infection of naive salmon exposed to salmon farms known to be infected with PRV, I think we are all coming into agreement that PRV is spreading from salmon farms and infecting wild salmon, with increasingly understood potential to cause disease.

**If DFO hopes to regain public trust**, I believe a primary ingredient will be support from the growing non-government research community who are examining PRV, as well as, the environmental groups who have a 25+ year history with the salmon farming industry. It is alarming the Aquaculture Management Division is making these decisions in absence of a division whose sole focus is survival of wild salmon.

**I hope you will find a path to initiate PRV screening and prohibit transfer of farm salmon infected with PRV as per the law.** The public expects this and it would motivate the industry to meet this bar. As it stands now you appear to be lowering the bar so that they can continue spreading aquaculture-source PRV.

I recognize that no minister has taken a strong regulatory position with this industry, but the difference now is that you are presiding over the sum of DFO failure and an extreme state of collapse in wild salmon in BC today. DFO is not doing the job we are paying for.

You have the power to do the right thing. This coast needs a minister who can stand up to the 4 companies making a profit from this. I remain willing to help.

All the best,



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Yellow salmon in Creative Salm

**Pages 863 to / à 864  
are duplicates of  
sont des duplicatas des  
pages 855 to / à 856**

## Miller-Saunders, Kristi

---

**From:** Gideon Mordecai <gmordecai@eoas.ubc.ca>  
**Sent:** June-27-19 12:51 PM  
**To:** Miller-Saunders, Kristi  
**Cc:** Schulze, Angela  
**Subject:** Re: Slides for presentation  
**Attachments:** M2.pdf; S1.pdf

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Gid

**M2**

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-----Original Message-----

From: Gideon Mordecai <[gморdecai@eoas.ubc.ca](mailto:gморdecai@eoas.ubc.ca)>

Sent: June-26-19 2:42 PM

To: Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>

Subject: Slides for presentation

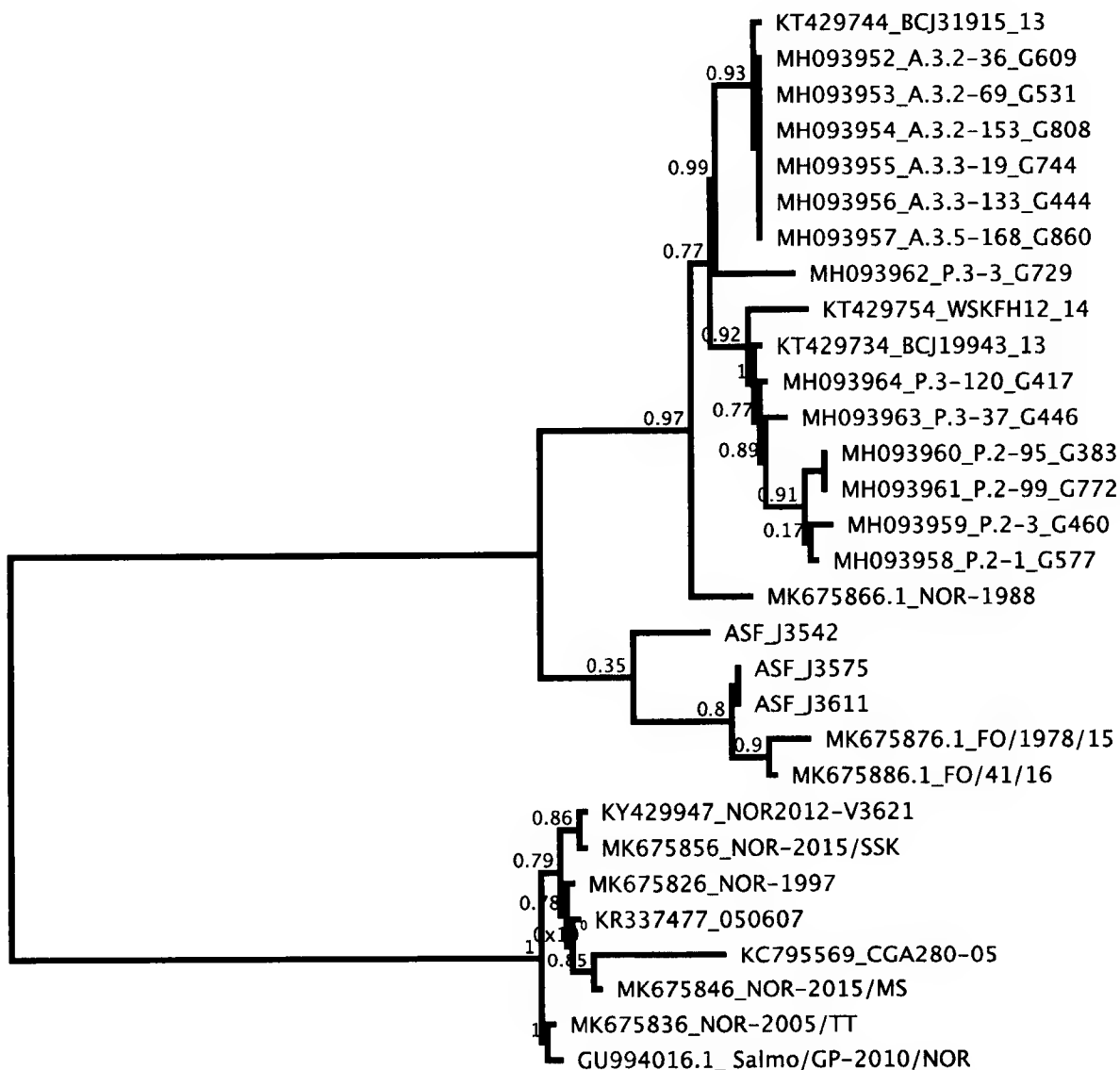
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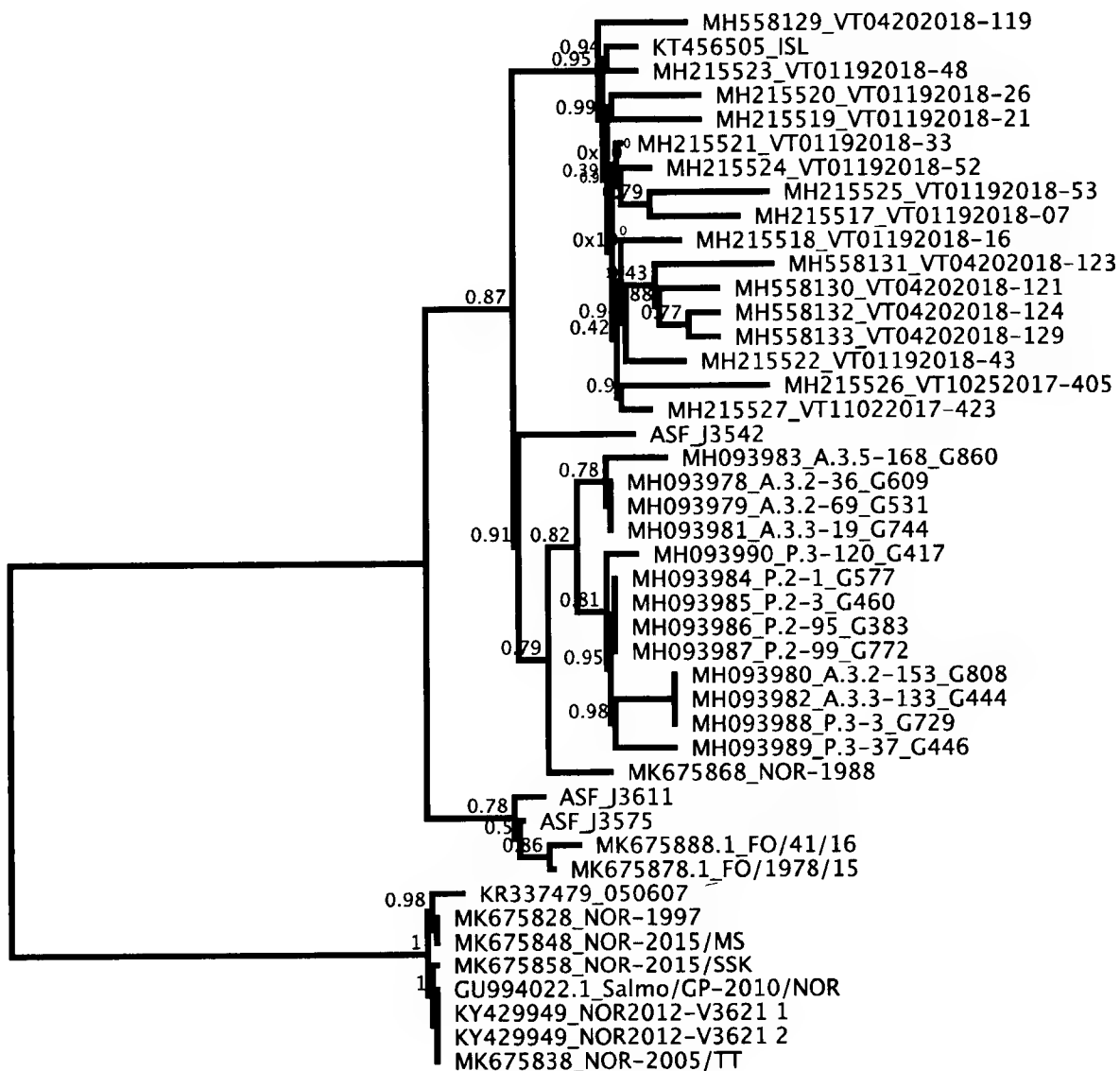
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0.003



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**Sent:** June-27-19 3:13 PM  
**To:** Miller-Saunders, Kristi  
**Cc:** Gideon Mordecai; Schulze, Angela  
**Subject:** Re: Slides for presentation

Oh dear. This complicated the story. I can reach out to Ian. I'm at a conference social but can also look at the original file from Ian to see if country was included in the designation.

-Amy

On Jun 27, 2019, at 5:00 PM, Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)> wrote:

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**Sent:** June-27-19 3:44 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re: Slides for presentation  
**Attachments:** PRVS1.pdf

Do you want it like this or as a multi fasta file. In the image attached, nucleotide substations to the icelandic sequence (top) are highlighted.

On 27 Jun 2019, at 15:25, Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)> wrote:

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I don't think I included the Faroe islands in Amy's trees. Maybe not too late to change, but not sure if this is important to Amy's conclusions... (Sorry Amy!).

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Yes, and this is also corroborated by the Dhamothoran tree, but this is a Norwegian sample from 1988 - in case that is important. Maybe the PRV strain we have in BC is more similar to the older Norwegian strain as this is closer to when it was introduced?

**"are the other sequences within this cluster Norwegian sequences or what?:** The MH2155XX samples are all from escaped Atlantic salmon from Washington state (see this

paper: <https://virologyj.biomedcentral.com/articles/10.1186/s12985-019-1148-2>). The MH558XX samples are also escapees caught by angels in the Skagit river from the same paper. I guess these are the kind of thing which the minister is trying to detect?

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**Sent:** June-27-19 4:13 PM  
**To:** Miller-Saunders, Kristi  
**Subject:** Re: Slides for presentation  
**Attachments:** S1\_with\_colour.pdf

So you want something like this?

Dark blue = Norway  
Light blue = Iceland  
Red = Canada  
Orange = Faroe Islands

Also, from your edits to the paper, I guess you want to include Emiliano's in-situ images. I have not heard back from Emiliano. Is he happy with that situation?

On 27 Jun 2019, at 16:03, Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)> wrote:

The escaped Washington fish were Icelandic origin, so they should be color coded with the Icelandic sequence

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I need to see both alignments and trees that include the Icelandic sequences. Do not bother including PRV-2 or -3. The purpose is to determine what segments would be required to distinguish the Icelandic strain from the BC variants currently detected. The department is embarking on a new PRV testing program that seeks to monitor hatchery fish to ensure that new variants of PRV are not being introduced into BC waters. As eggs for the industry have historically been from Norway and Iceland, it is the variants in these countries of specific interest. The latest phylogenetic study out of Espen's lab shows that the Norwegian HSMI-PRV (1b) is easily recognized via segment M2 and S1 and we need to know if the Icelandic strain could also be differentiated using these segments alone

I will send slides in another email. Sorry, I forgot

Kristi Miller-Saunders, PhD  
Head, Molecular Genetics  
Pacific Biological Station  
3190 Hammond Bay Rd  
Nanaimo BC V9T 6N7  
250-756-7155  
[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)

-----Original Message-----

From: Gideon Mordecai  
<[gmordecai@eoas.ubc.ca](mailto:gmordecai@eoas.ubc.ca)>  
Sent: June-26-19 2:42 PM  
To: Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>  
Subject: Slides for presentation

Hi Kristi,

Could you please send along any summary slides for the SSHI for my presentation next week. I want to show the breadth of work that our group is working on before I get into the virus story.

Many thanks,

Gideon

Ps. I spoke to Angela today and I am happy to make the PRV tree you requested. I am waiting for Angela to send me the selection of sequences. Let me know if there is anything in particular you want highlighted on the tree... Angela mentioned something about the Icelandic sequences.





## Miller-Saunders, Kristi

---

**From:** Amy Teffer [REDACTED]  
**Sent:** June-27-19 5:59 PM  
**To:** Miller-Saunders, Kristi  
**Cc:** Gideon Mordecai  
**Subject:** Re: Slides for presentation

I'm catching up on what all of this means and whether it is worth adjusting our conclusions. My first thought is yes, because if we add more samples and our conclusions change, I don't like that. And I really don't want more controversy over this paper than we are likely to get anyway ; )

I'll think on this and will make a decision by tomorrow if we need to adjust the tree (include newer sequences) and our conclusions (escapee sequence similarities). It means more work for me and Gid. Gid - do you have time to chat about this tomorrow on the phone?

On Thu, Jun 27, 2019 at 7:54 PM Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)> wrote:

I think this will work for what I needed. But if there were something to change it would be to add more Norwegian PRV-1a sequences in, as there is only 1. Need to see how interspersed they are now that we have a good sample size from Iceland and other areas. More really for Amy's benefit (i.e. her paper), not that I am saying it needs to change. Amy?

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics

Pacific Biological Station

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[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)

**From:** Gideon Mordecai <[gмордеcai@eoas.ubc.ca](mailto:gмордеcai@eoas.ubc.ca)>  
**Sent:** June-27-19 4:30 PM  
**To:** Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>  
**Subject:** Re: Slides for presentation

Ok, when is the meeting? [REDACTED] I will be working this evening and tomorrow if you need any other resources. I won't be available this weekend or Monday, [REDACTED] so any changes will have to happen tomorrow, Tuesday or Wednesday.

Gid

On 27 Jun 2019, at 16:24, Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)> wrote:

This is perfect. There are three nucleotides that separate the Icelandic sequences in S1, so relatively easy job.

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics

Pacific Biological Station

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Nanaimo BC V9T 6N7

250-756-7155

[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)

**From:** Gideon Mordecai <[gмордеcai@eoas.ubc.ca](mailto:gмордеcai@eoas.ubc.ca)>

**Sent:** June-27-19 3:44 PM

**To:** Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>

**Subject:** Re: Slides for presentation

Do you want it like this or as a multi fasta file. In the image attached, nucleotide substations to the icelandic sequence (top) are highlighted.

On 27 Jun 2019, at 15:25, Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)> wrote:

Can you send this to me aligned? I don't have alignment software on my computer and I don't know where Angela is.

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics

Pacific Biological Station

3190 Hammond Bay Rd

Nanaimo BC V9T 6N7

250-756-7155

[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)

**From:** Gideon Mordecai <[gмордеcai@eoas.ubc.ca](mailto:gмордеcai@eoas.ubc.ca)>

**Sent:** June-27-19 2:57 PM

**To:** Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>

**Cc:** Schulze, Angela <[Angela.Schulze@dfo-mpo.gc.ca](mailto:Angela.Schulze@dfo-mpo.gc.ca)>; Amy Teffer <[akteffer@gmail.com](mailto:akteffer@gmail.com)>

**Subject:** Re: Slides for presentation

Your questions in bold and my replies below.

**"Did we have Faroe island samples in our previous analysis?"**

I don't think I included the Faroe islands in Amy's trees. Maybe not too late to change, but not sure if this is important to Amy's conclusions... (Sorry Amy!).

**"it looks like the BC fish are more similar to wt Norway (MK675866) than to the east coast".**

Yes, and this is also corroborated by the Dhamothoran tree, but this is a Norwegian sample from 1988 - in case that is important. Maybe the PRV strain we have in BC is more similar to the older Norwegian strain as this is closer to when it was introduced?

**"are the other sequences within this cluster Norwegian sequences or what?:** The MH2155XX samples are all from escaped Atlantic salmon from Washington state (see this paper: <https://virologyj.biomedcentral.com/articles/10.1186/s12985-019-1148-2>). The MH558XX samples are also escapees caught by anglers in the Skagit river from the same paper. I guess these are the kind of thing which the minister is trying to detect?

**Can I see if there are any fixed differences in our BC sequences compared with the Iceland sequences**

Site 596 is different in the Icelandic sequence to all others (including the WA escapees). Alignment attached.

On 27 Jun 2019, at 14:00, Miller-Saunders, Kristi  
<[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)> wrote:

Well that is interesting. The ASF sequences now all look most similar to the Faroe Islands, half way between Greenland and Norway, especially those sampled in Greenland. I wonder whether the "European" fish could be from the Faroe Islands". Amy that is something we may want to check with Ian Bradbury about, whether his test can distinguish that. So in this analysis, it looks like the BC fish are more similar to wt Norway (MK675866) than to the east coast, which kind of wrecks my theory! Did we have Faroe island samples in our previous analysis?

For the sake of this exercise on the Minister's PRV testing, we are interested in distinguishing Island from BC, which is within the top S1

cluster in your analysis; are the other sequences within this cluster Norwegian sequences or what? If Norwegian, why is MK675868 so different (the 1988 wt Variant before HSMI outbreaks).

For S1 anyway, if that is all that is available, can I see if there are any fixed differences in our BC sequences compared with the Iceland sequences—i.e. can you send the alignments and better demarcate BC vs Norway samples?

*Kristi Miller-Saunders, PhD*

Head, Molecular Genetics

Pacific Biological Station

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[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)

**From:** Gideon Mordecai <[gмордеcai@eoas.ubc.ca](mailto:gмордеcai@eoas.ubc.ca)>

**Sent:** June-27-19 12:51 PM

**To:** Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>

**Cc:** Schulze, Angela <[Angela.Schulze@dfo-mpo.gc.ca](mailto:Angela.Schulze@dfo-mpo.gc.ca)>

**Subject:** Re: Slides for presentation

Hi Kristi,

Attached are trees for M2 and S1. I hope this is the kind of thing you wanted? I can easily add/ remove sequences, colour a certain clade, or make some of the sequence names bold etc... if you need.

I rooted the trees, and swapped siblings to try and emulate Dhamotharan et al. 2019 (figure also attached) so you can easily compare.

I added 'ISL' to the end of the Icelandic sequence name. I could only find an S1 sequence for the Icelandic strain. Do you have more info for the rest of the genome and do you need trees for the other segments?

Gid

<image001.png>

On 26 Jun 2019, at 15:53, Miller-Saunders, Kristi  
<[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)> wrote:

I need to see both alignments and trees that include the Icelandic sequences. Do not bother including PRV-2 or -3. The purpose is to determine what segments would be required to distinguish the Icelandic strain from the BC variants currently detected. The department is embarking on a new PRV testing program that seeks to monitor hatchery fish to ensure that new variants of PRV are not being introduced into BC waters. As eggs for the industry have historically been from Norway and Iceland, it is the variants in these countries of specific interest. The latest phylogenetic study out of Espen's lab shows that the Norwegian HSMI-PRV (1b) is easily recognized via segment M2 and S1 and we need to know if the Icelandic strain could also be differentiated using these segments alone

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-----Original Message-----

From: Gideon Mordecai  
<[gmordecai@eoas.ubc.ca](mailto:gmordecai@eoas.ubc.ca)>  
Sent: June-26-19 2:42 PM  
To: Miller-Saunders, Kristi  
<[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)>  
Subject: Slides for presentation

Hi Kristi,

Could you please send along any summary slides for the SSHI for my presentation next week. I want to show the breadth of work that our group is working on before I get into the virus story.

Many thanks,

Gideon

Ps. I spoke to Angela today and I am happy to make the PRV tree you requested. I am waiting for Angela to send me the selection of sequences. Let me know if there is anything in particular you want highlighted on the tree... Angela mentioned something about the Icelandic sequences.

--

><=> ><=> ><=>

Amy K. Teffer, PhD



s.19(1)



## Miller-Saunders, Kristi

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**From:** [REDACTED]  
**Sent:** June-28-19 6:26 AM  
**To:** Miller-Saunders, Kristi  
**Cc:** Gideon Mordecai  
**Subject:** Re: Slides for presentation

Thanks Kristi. That makes sense. I'm just getting to my office to see what the resolution of Ian's genotyping was, and if that doesn't include country of origin then I can reach out to him whether that info exists for these fish. We don't mention Faroe Islands in the paper and maybe that could be as far as we go for this manuscript (ie mention that we cannot say whether the strain is from Faroe or Norway). Anyway, I'll think about this today.

Gid, let's still plan to chat.

-Amy

On Jun 28, 2019, at 8:57 AM, Miller-Saunders, Kristi <[Kristi.Saunders@dfo-mpo.gc.ca](mailto:Kristi.Saunders@dfo-mpo.gc.ca)> wrote:

I am not sure how much your conclusions will change, as the new trees did not have enough representation from Norway (for PRV-1a) to really make a conclusion on that. However, the closeness of the two Greenland samples to the Faroe Islands PRV seems highly supported, and the most recent paper out of Rimstad's group also shows this distinction with the Faroe Islands. That said, Faroe islands is European, so it is not unfathomable that the Atlantic salmon of "European" origin could come from there. So...the movement of PRV from Europe to the east coast of North America is not an incorrect hypothesis based on these trees, but whether it came from Norway, or the Faroe Islands, is in question. More Norwegian sequences in the tree while also including Faroe, as well as at least a subset of the Icelandic sequences will be important. If you don't want to go to a lot of rethinking on this, you could leave the hypothesis out about movement from the east coast to west coast of North America, and that is something that Gid can follow up in the more intensive phylogenetic paper he will write (with way more west coast sequences).

---

**From:** [REDACTED]  
**Sent:** June 28, 2019 4:18 AM  
**To:** Gideon Mordecai  
**Cc:** Miller-Saunders, Kristi  
**Subject:** Re: Slides for presentation

Thanks! I'm available all day today so just let me know when works for you.

-Amy

On Jun 27, 2019, at 10:15 PM, Gideon Mordecai <[gmordecai@eoas.ubc.ca](mailto:gmordecai@eoas.ubc.ca)> wrote:

s.19(1)

Yes let's chat, and yes it's not much work and I am happy to help.

On 27 Jun 2019, at 17:58, Amy Teffer <[REDACTED]> wrote:

I'm catching up on what all of this means and whether it is worth adjusting our conclusions. My first thought is yes, because if we add more samples and our conclusions change, I don't like that.

## Miller-Saunders, Kristi

---

**From:** Miller-Saunders, Kristi  
**Sent:** June-28-19 1:40 PM  
**To:** Yovela Wang  
**Cc:** Hinch, Scott  
**Subject:** manuscript  
**Attachments:** YW\_manuscript APRIL 23 SH\_KM.docx

Hi,  
Finally got a chance to complete my review and comments on the paper. This is coming together nicely,

Emiliano and I worked on the histo section together.

I think it is still on the long side and I am not loving the last paragraph, but perhaps someone else can keep working on that. I may take another look at the last few paragraphs and provide more feedback in the coming week.

Once you have incorporated my changes, and shown them to Scott, you should circulate to co-authors. I need to contact Marc Trudel and give him the heads up on this paper, and he needs to be a co-author, as does Terry, and especially Hugh—who was actually the person who ready your histopathology. Before you circulate, please have it in complete manuscript form (need institutional addresses, acknowledgements, etc.. and an idea of where you intend to submit it. Clearly you will need to format it for whatever that journal might be. I think I would stay away from the disease-focused journals and again go to Plos or Facets or something of that nature. disease-focused journals are most always wary of the different way that we approach fish health science, although your paper is closer to their methods than most.

Kristi

---

**From:** Yovela Wang  
**Sent:** December 24, 2018 10:02 PM  
**To:** Miller-Saunders, Kristi  
**Cc:** Hinch, Scott  
**Subject:** Re: (Help) Fish sample collection information

s.19(1)  
s.21(1)(a)  
s.21(1)(b)

Hi Kristi,

Thanks for the prompt reply!

Yovela

On 2018-12-24, 9:56 PM, "Miller-Saunders, Kristi" <Kristi.Saunders@dfo-mpo.gc.ca> wrote:

Prior to 2014 they did not issue animal care certificate numbers as all fish were under a universal DFO scientific collection assessment permit. I suggest you simply copy strahan's statement.

Kristi

From: Yovela Wang [REDACTED]  
Sent: December 24, 2018 9:27 PM  
To: Miller-Saunders, Kristi  
Cc: Hinch, Scott  
Subject: (Help) Fish sample collection information

Hi Kristi,

[REDACTED]

When I was trying to submit my thesis, the library required me to include animal care or other appropriate approval certificate numbers in the preface. Since my fish were caught by DFO, I wonder if you could help me with finding this information?

I noticed that in Tucker et al 2018, he wrote:

"Juvenile salmon collected under a scientific fishing permit (MECTS # 2014-502-00249) issued to Pacific Region Department of Fisheries and Oceans (DFO) staff by the Government of Canada, DFO, Regional Director Fisheries Management. This work does not require an animal care protocol pursuant to an exemption contained in the Canadian Council on Animal Care (CCAC) guidelines applying to fish lethally sampled under government mandate for assessment purposes (4.1.2.2)"

Is that the same situation with my fish? They were caught from 2012 to 2014. I assume they were caught under different fishing permits? Do you have the permit numbers?

Thanks.

[REDACTED]

Yovela

s.19(1)

**Pages 894 to / à 988  
are withheld pursuant to sections  
sont retenues en vertu des articles**

**21(1)(b), 13(1)(c), 21(1)(a)**

**of the Access to Information Act  
de la Loi sur l'accès à l'information**

**Miller-Saunders, Kristi**

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**From:** Emiliano Di Cicco <emiliano.dicicco@unicam.it>  
**Sent:** June-30-19 10:00 AM  
**To:** [REDACTED] Miller-Saunders, Kristi  
**Subject:** Re: Message from the Minister

I'm available, if required.

As foreign researchers... Espen Rimstad has been already included in the PRV CSAS... [REDACTED]  
[REDACTED]

There could be also [REDACTED]

Both Espen [REDACTED] are veterinarian from Norway.

I would obviously include Hugh Ferguson in this list , [REDACTED]  
[REDACTED]

Emiliano

On Sun, Jun 30, 2019, 08:06 [REDACTED] wrote:

Could you please recommend a few (2-3) names for the Minister's Advisor committee on Aquaculture science. Particularly interested in European researchers with experience in fish health (viruses & sea lice) and a Canadian (including if you would participate). This is now urgent, thanks.

[REDACTED]

Begin forwarded message:

**From:** [REDACTED]  
**Date:** June 29, 2019 at 12:20:23 PM PDT  
**To:** [REDACTED]  
[REDACTED]  
**Subject:** Message from the Minister

I received a reply from Minister Wilkinson yesterday to my offer to have WSF put together a couple of briefing sessions for him, one on sea lice and second on PRV. He replied that he would rather I submit nominations to his Advisory Committee on Aquaculture Science. I replied that my two nominees for the Canadian positions were the two of you, and that I would consult with you about others, particularly as regards the proposed two foreign positions. [REDACTED]

[REDACTED] I would favor looking for ways that Kristi, as a DFO employee, could be a DFO attendee in some form or other.

My own suggestions for foreign names would likely include [REDACTED] and a Norwegian scientist (but who?). I'm sure you would have others.

mentioned that there were likely some changes coming to the proposed make-up of the committee - I can easily imagine that the farmers would want [redacted] to be included and might push for more Canadian participants, at least one of whom was a BCSFA nominee. I mentioned in my email to the Minister that I had heard that changes had been proposed and asked for an elaboration if he could give it.

I think I should reply ASAP. Do you think you could circulate your suggestions, with the idea that we might have a short phone meeting after we had all the names on the list by the end of the week?

[redacted] please feel free to join us in any way you see fit.

5 per mille  
Università di Camerino

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Aspirante	Aspirando	Importo	Codice fiscale

SCelta PER LA DESTINAZIONE DEL CINQUE PER MILLE

IMMANDANDO SULLA BUSTA IDENTIFICA E PAGA UNIVERSITÀ

*Claudio Peltinari*

Importo 5 per mille: 16.810,43

Sulla ricerca UNICAM, ci metto la *firma*.

s.19(1)